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MANUAL OF CLINICAL MICROSCOPY AND CHEMISTRY

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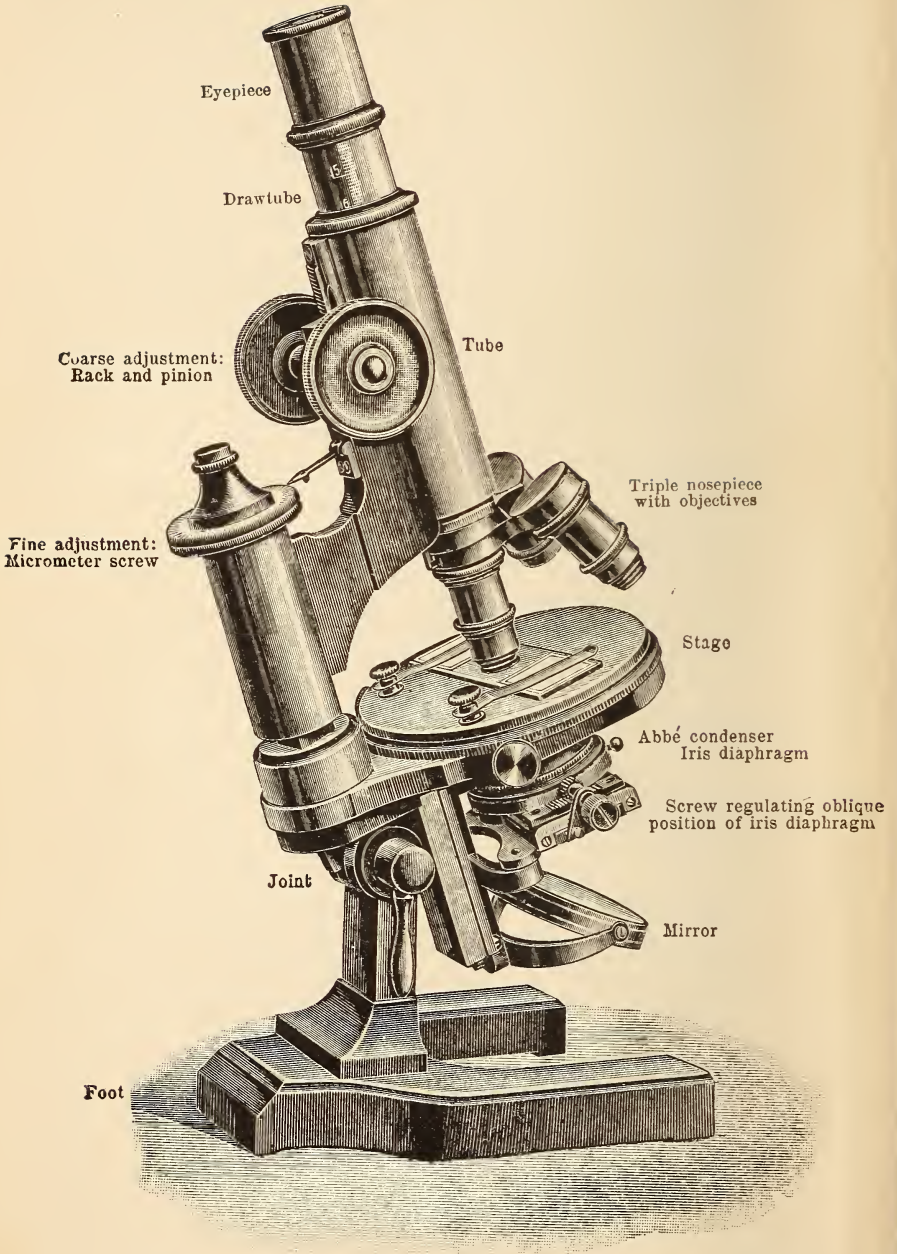

[Leitz Continental Microscope with Adjustable Revolving Stage.]

MANUAL

OF

CLINICAL MICROSCOPY AND CHEMISTRY

PREPARED FOR THE USE OF STUDENTS AND PRACTITIONERS OF MEDICINE

BY

DR. HERMANN LENHARTZ

PROFESSOR OF MEDICINE AND DIRECTOR OF HOSPITAL AT HAMBURG, ETC., ETC.

AUTHORIZED TRANSLATION FROM THE FOURTH AND LAST GERMAN EDITION, WITH NOTES AND ADDITIONS

BY

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WITH 148 ILLUSTRATIONS IN THE TEXT AND 9 COLORED PLATES

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TO

DANIEL BENNETT ST. JOHN ROOSA, M.D., LL.D.

PRESIDENT OF THE NEW YORK POST-GRADUATE MEDICAL SCHOOL
AND HOSPITAL, EX-PRESIDENT OF THE NEW YORK
ACADEMY OF MEDICINE, ETC.

IN RECOGNITION OF HIS MANY ATTAINMENTS AND CEASELESS EFFORTS
TO ELEVATE THE SCIENTIFIC AND ETHICAL STATUS OF THE
MEDICAL PROFESSION IN AMERICA, THE AMERICAN
EDITION OF THIS WORK IS

RESPECTFULLY DEDICATED

BY THE

TRANSLATOR

TRANSLATOR'S PREFACE.

THE great favor accorded to three German editions of this well-known work, the simplicity in arrangement and presentation of the subject-matter, and the adaptability to the needs of the general practitioner and undergraduate student have induced me to undertake, amidst many other arduous duties, the rather laborious task of preparing an English edition—a task which only those who have had a similar experience can really appreciate. The more adequately to meet the requirements of the busy general practitioner and specialist, whose time is too much occupied with the routine of medical practice to consult larger, more complex, and technical treatises on the subject, I have, wherever it seemed to me necessary, inserted notes and illustrations which to the expert may appear superfluous. As regards these additions, ten years' experience in teaching many hundreds of graduate students of medicine has convinced me of the necessity of emphasizing points in examinations which might, at first glance, seem to be self-evident. All such additions are indicated by brackets [ ]. Furthermore, the value of the present edition has been greatly enhanced by notes and additions, kindly furnished by the author in advance from the new fourth German edition, shortly to be issued. Among the author's additions are sections on the molecular concentration of the blood and urine (cryoscopy), the bacillus dysenteriæ (Shiga), the paratyphoid bacillus (Schottmüller), a new method for staining the blood, and addenda to the section on the Widal reaction. This edition, therefore, reflects the essence of nearly a quarter of a century of practical experience of one of the foremost diagnosticians of Germany—the country in which diag-

nosis by laboratory methods has become an art. Notwithstanding the elementary character of the book, the different subjects have been so carefully prepared by the author that I may be pardoned for assuming that even the professional laboratory worker may now and then find a hint which will aid him to the solution of some problem. A copious index has been added to facilitate ready reference.

In conclusion, I wish to thank the F. A. Davis Company for the courtesy and consideration they have shown to me, and Drs. H. B. Sheffield and S. D. Jacobson,—the former for unselfish aid in proofreading and suggestions, the latter for aid in certain portions of the text.

H. T. B.

New York, February, 1904.

PREFACE TO THE THIRD EDITION.

To MY great satisfaction my text-book met with such wide favor after my departure from the University of Leipzig that it can now appear in a third edition.

I have submitted it to a thorough revision in accordance with the present *status* of our science. The number of figures in the text has been increased, and several illustrations of the two previous editions have been replaced by new ones. Four new illustrations (acute leukemia and malaria) have also been added to the colored plates.

May the present edition serve to disseminate the methods of examination discussed!

HERMANN LENHARTZ.

PREFACE TO THE FIRST EDITION.

It was my endeavor to offer to physicians and students a book which would not only instruct them in clinical microscopic and chemic *methods of examination*, but also aid them in interpreting their *diagnostic significance* in practice. For some time routine exercises bearing upon our subject have but seldom been carried out in the majority of universities, and consequently their practical value has been underestimated by the practitioner. Their application, however, is daily growing more necessary, the more the material relative thereto increases. This has already become too voluminous to be adequately treated in a clinical or propedeutic manner. It is only by *practical exercises*, such as have long been customary in other sciences, that students can acquire the knowledge requisite in practice. From this standpoint I have for many years arranged and conducted this special course in the Leipzig clinic.

The lectures here given in augmented form are the result of my work as a teacher. The abundant material of this clinic, with which I have been almost constantly associated since the beginning of my assistantship under the guidance of Ernst Wagner in 1879, has afforded me the opportunity, especially in recent years, to devote particular attention to the subjects here discussed. I should not, therefore, omit to thank Geheimrath Curschmann for the clinical material which he placed at my disposal.

A glance at the table of contents will show the divisions of the book. It may also be remarked that in the *microscopic* part I have considered only the examination of fresh and dried impression and teased preparations, because the more complicated

examination of sections, etc., belongs to the domain of pathologic anatomy. The microscopic description has in all instances been preceded by a careful *macroscopic résumé.*

Chemistry has received detailed consideration especially in connection with examination of the urine, while in those sections devoted to the study of the blood and gastric contents only the practical tests, including forensic examination of blood-stains, have been discussed.

In the *first* section of the book the vegetable and animal parasites have been briefly dealt with. In this way only was it possible uniformly to present parasitology in its important bearing upon pathologic processes, and to avoid frequent repetitions in the subsequent sections. The greater space devoted to a description of the vegetable parasites can readily be understood at the present day. As regards many questions in this connection, I have consulted especially Baumgarten's "Mykologie" and Leuckart's classical treatise on parasitology, which reflect the accumulated knowledge in this domain.

In *examinations of the blood* the studies pursued by Ehrlich and others upon analytic staining have been thoroughly discussed; that many questions in this direction still await explanation will be admitted by anyone familiar with the subject.

The discussion of the *sputum* and *urine* has, I believe, been handled in a comprehensive, though somewhat brief, manner; here, however, I have constantly kept in mind the interests of the practitioner, and have, therefore, in all instances thoroughly considered questions of diagnostic importance.

Owing to the text-book character of the treatise, I have omitted all references to literature; on the other hand, however, when of historic interest, I have included in the text the names of authors who have elaborated the subjects.

HERMANN LENHARTZ.

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MANUAL OF CLINICAL MICROSCOPY AND CHEMISTRY.

INTRODUCTORY REMARKS.

CONSTRUCTION AND MANIPULATION OF THE MICROSCOPE—NECESSARY REAGENTS AND APPARATUS.

I. THE *optic portion of the microscope* is composed of the *objective*, which is screwed into the lower end of the tube, and the ocular [or eyepiece (see Figs. 1, 2, and 3, *G*)] which is inserted into the upper opening. The objective gives but a magnified inverted image, which is received and further enlarged by the ocular. The first [the objective

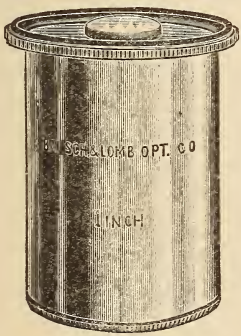


Fig. 1.—Eyepiece.

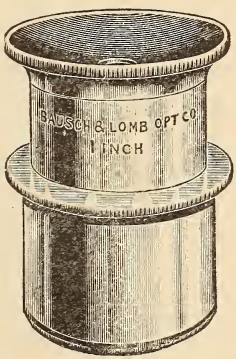


Fig. 2.—Eyepiece.

(see Fig. 3, *F*, and Figs. 7, 8, and 9)] consists of a system of crown-glass converging and flint-glass diverging lenses which permit as much as possible the exclusion of *chromatic aberration*. The latter would become manifest with the use of but *one* lens by a greater or less reduction and disturbance of the field of vision through the appearance of a colored marginal zone, because the rays composing the white light would not be uniformly refracted. A further source of disturbance of the image—namely, *spheric aberration*—is overcome by a diaphragm placed within the tube. This arrests the *peripheral* rays of the cone of light which passes through the objective, and brings the central rays to a focus.

The field of usefulness of the microscope has been greatly extended in recent years by the introduction of *immersion lenses* and the Abbé *condensing apparatus*. In the use of "dry" lenses, which in former

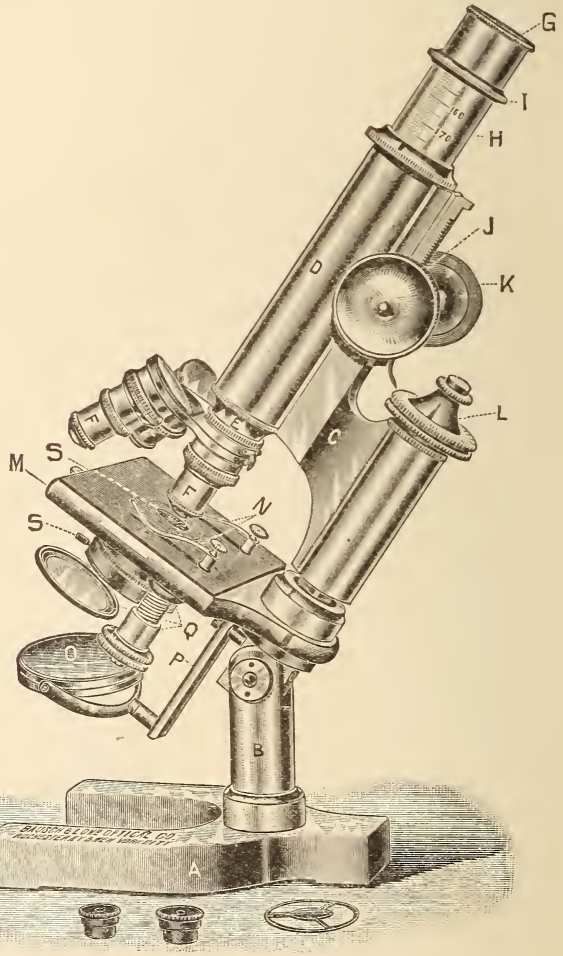


Fig. 3.—Bausch & Lomb BB Continental Microscope with Fixed Stage. (For explanation of letters see frontispiece.)

years were the only ones employed, the light reflected from the plane or concave mirror is constantly subject to loss. This is due to the difference in refractive index of the media to be penetrated, such as the

slide, cover-glass, and varying stratum of air between the preparation and front lens, so that the rays of light always suffer partial refraction in their passage to the objective. In a great many instances, especially in the study of the pathogenic bacteria, the assistance offered by the microscope is decidedly reduced by this loss of light. Through the introduction of immersion lenses into microscopy by Koch the loss of light is reduced to a minimum. The interposition of water between the front lens and the preparation had formerly offered many advan-

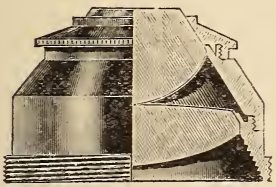


Fig. 4.—Double Abbé Condenser.

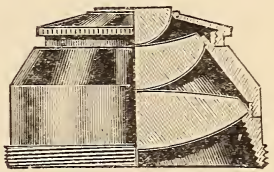


Fig. 5.—Triple Abbé Condenser.

tages. A much greater increase in the volume of light and in the distinctness and clearness of the image is, however, secured by the use of an immersion fluid which, like cedar-oil, possesses the same refractive index as crown glass; by this means all refraction of the rays of light before they enter the objective is overcome. The value of immersion lenses is greatly enhanced by the *Abbé condenser*. The latter consists of a combination of two [double condenser] or three lenses [triple con-

Fig. 6.—Iris Diaphragm.

denser]. In the double condenser the first [top] lens is plano-convex, and the second [bottom] biconvex [see Fig. 4]; in the triple condenser there is, in addition to the two lenses just mentioned, a middle concavo-convex lens [see Fig. 5]. The condenser is so placed in the collar beneath the microscope stage that the flat surface of the upper plano-convex lens is level with the stage. A powerful cone of light can then be concentrated upon the specimen. The intensity of the light is regulated by a series of concentrically perforated disks placed in the dia-

phragm-holder. The light is more conveniently controlled by means of the so-called "*iris diaphragm*" [placed under or over the condenser or both (see Fig. 3, *S*)], which permits ready and rapid change in the size of the diaphragm at any moment.

[For durability the cylinder-diaphragm attachment with removable caps is preferable. These removable caps are made by Leitz, Spencer Lens Company, Bausch & Lomb, and Queen & Co., with three sizes of aperture. For years the Leitz microscope II C, made for the American trade, has been equipped with this form of diaphragm only, the iris being confined to the under surface of the separate Abbé condenser attachment. It is only recently that they have introduced the iris on the stage of the II C, and this outfit is supplied on special order. For many years the Bausch & Lomb Optical Company used only the so-called "thimble" or "cap" diaphragm; they then attached the iris to the stage, but their new BBS models are also supplied with the cylinder diaphragm.

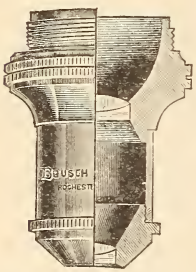


Fig. 7.—Two-thirds Objective.

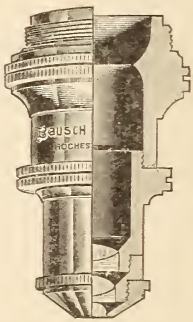


Fig. 8.—One-sixth Objective.

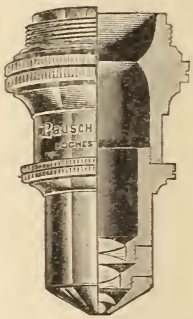


Fig. 9.—One-twelfth Oil-Immersion Objective.

Many physicians from various parts of the country have expressed to us much dissatisfaction with the iris permanently fixed to the stage. In the routine microscopic work at the New York Post-graduate Laboratory instruments with such attachments were long ago discarded, because, when specimens of liquids, such as urine (which is most often examined by the physician), were examined by the slide method, now exclusively in use there, the leaves of the diaphragm became wet, subsequently rusted, and were thus soon rendered useless. Furthermore, too hasty or forcible lowering of the tube may shatter the iris diaphragm by contact with the metal casing of the objective. None of these objections applies to the cap diaphragm, and it has been our experience, as well as that of many others we have consulted, that proper manipulation of the substage screw for lowering and raising the diaphragm accomplishes more than can be secured by increasing or decreasing the size of the aperture of an iris diaphragm fixed to the stage. This, we have

found, is particularly true with regard to searching for delicate, small hyaline and finely granular casts in clear urines with almost invisible sediments.—Brooks.]

II. In the *selection of a microscope* the price of the instrument is an important question. Although it is, as a rule, advisable not to be sparing in outlay, it should be noted that, for the practitioner who does not intend to make a special study of the bacteria, a simple microscope ranging in price from $45 to $50 is quite sufficient. For this amount he can obtain a firm stand (Leitz [Bausch & Lomb, Spencer, or Queen]),

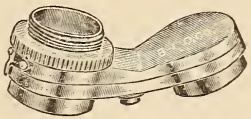


Fig. 10.—Double Nosepiece.

with oculars I and III [1- and 2-inch] and objectives 3 and 7 [$^2/_3$ and $^1/_6$], by means of which magnifications up to 500 diameters can be secured. Besides the examination of the sputum, urine, and other secretions, examination for the tubercle bacillus and, with a little practice, even the gonococcus can be successfully conducted. [While it is true that the tubercle bacillus, gonococcus, and other bacteria *can* be seen with dry objectives of the powers mentioned, we would strongly advise against the use of such by the novice except where unavoidable.—Brooks.]

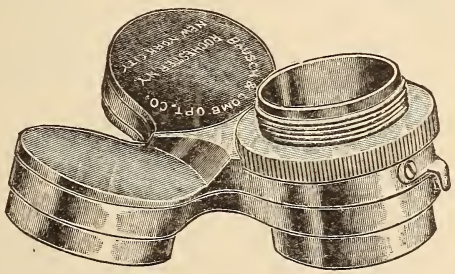


Fig. 11.—Triple Nosepiece.

For all those to whom *price* is not a pressing consideration, it is strongly advisable to choose a better outfit, especially a good stand with coarse adjustment for moving the tube. [Also a double or triple nosepiece (see Fig. 3, *E;* and Figs. 10 and 11) for lenses of low, medium, and high magnifications.—Brooks.] The purchase of better lenses, particularly an immersion lens, can subsequently be made. Unexcelled microscopes are manufactured by C. Zeiss, of Jena, and E. Leitz, of Wetzlar [also by Bausch & Lomb, of Rochester (see Fig. 3); Spencer Lens Company, of Buffalo; and Queen & Co., of Philadelphia]. The illustrated

catalogues give all necessary information. In special scientific research, measuring and drawing apparatus, which can be attached to the microscope, are absolutely necessary. For the measurement of objects the

Fig. 12.—Micrometer Eyepiece Viewed from Below.

"micrometer ocular" can be recommended [Fig. 12]. With this attachment the eyelens can be accurately adjusted to any eye. For counting,

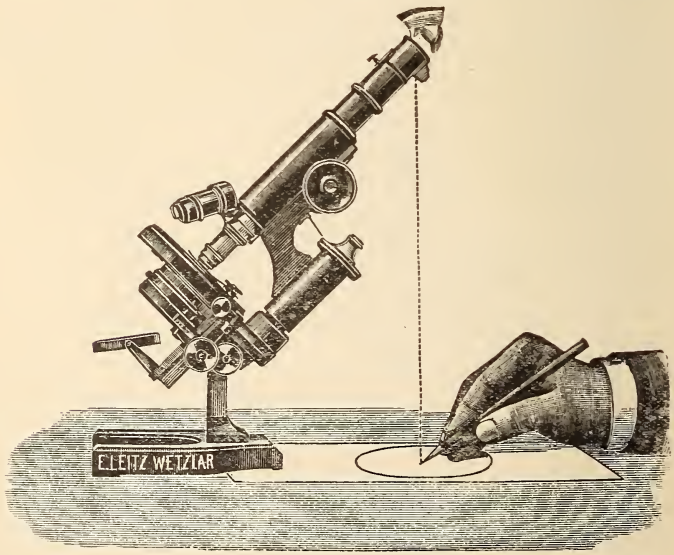


Fig. 13.—Drawing Eyepiece.

the "net micrometer," which is placed within the tube and rests upon the diaphragm, is also of value. For drawing, the camera lucida of Oberhäuser and Abbé or the drawing prism are chiefly employed [Fig.

13]. The author prefers the Abbé drawing apparatus, which is so arranged that the prism and mirror swing back from the eyepiece, permitting the use of the microscope for ordinary work and the changing of eyepieces without disturbing the camera lucida [see Fig 14]. By this means the different fields of the microscope can be uniformly examined. Some practice, however, is required in order to make satisfactory use of the apparatus. Not infrequently too great illumination is a disturbing element, and is not overcome by the smoked glasses which accompany the apparatus. In such instances embarrassment may be avoided by regulating the light by adjustment of the mirror. In this way sharp outline can be obtained upon the drawing surface, which is absolutely essential for drawing.

In the *apochromatic objectives* the chromatic and spheric aberration connected with the older objectives has been almost wholly eliminated by use of new kinds of glass and greatly improved methods of correction. The pictures appear wholly free of color, and by slight change of

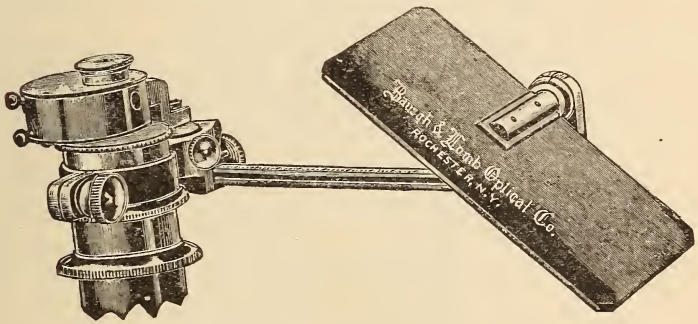


Fig. 14.—Camera Lucida.

the adjustment a uniform sharpness of the image is obtained at the center and periphery of the field.

III. In the *use of the microscope* the following rules should be observed: The instrument should be kept free of dust, and when frequently used it is advisable in the intervals to cover it with a bell-jar or to place it in the case.

In every examination it is, as a rule, best to begin with low magnification. Only after a general examination has been made should higher powers be employed (best with revolving nosepiece [see Fig. 3, *E*, and Figs. 10 and 11]). In using simple microscopes unprovided with *coarse adjustment* [see Fig. 3, *K*] focusing must be done by careful downward revolving movements of the drawtube, in order to avoid injury to the front lens from too strong advance of the objective. If the movement of the tube is obstructed or irregular, the tube should be cleansed with a little alcohol or slightly oiled.

Instruments provided with so-called rack and pinion permit ready

and safe approach of the objective to the preparation. In using immersion lenses a small drop of oil is placed upon the center of the cover-glass [or slide] and the lens carefully lowered [by means of the coarse adjustment] until it touches the oil. [Then by gentle manipulation of the fine adjustment the object can be brought into view.] After the use of the oil-immersion lens it should be freed of the excess of oil by gentle pressure with filter-paper and then wiped clean with a soft cloth [or absorbent cotton] moistened with pure benzin [or xylol]. Care should be observed not to use too much benzin [or xylol], as these, when in excess, soften the cement holding the lens in position. The *fine adjustment* [see Fig. 3, *L*] is under the control of the millimeter-screw, which, in modern instruments, is attached to the upper end of the supporting post. The right hand is used to work the fine adjustment, while the

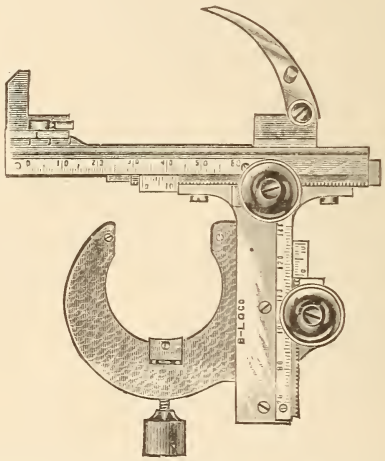


Fig. 15.—Bausch & Lomb Mechanical Microscope Stage.

left is employed to move the preparation in any direction upon the stage. [The fine adjustment is employed for *detail* work, and when in use should be kept in *constant motion*. Size, form, and depth are made out by its aid.

A mechanical stage (see Figs. 15, 16, and 17) is a valuable and labor-saving attachment. It is especially advantageous in the often tedious examinations for plasmodia of malaria, particularly of the estivo-autumnal forms; in ratio and enumeration counts of the blood-corpuscles; for gonococci in gonorrheal shreds; and for tubercle bacilli in the urine, feces, sputum, etc.—BROOKS.]

In the use of low powers light may be supplied with either the plane or concave mirror; with high powers the concave mirror, which concentrates more light, is usually employed [except with Abbé condenser, with which the plane mirror should always be used—BROOKS].

Daylight is generally to be preferred. Artificial light is best modified by a blue-glass plate or shoemaker's globe. [When electric light is used with a condenser, a frosted glass disk should be placed in the ring-holder which swings outward immediately beneath the condenser iris diaphragm. Many condensers, when level with the stage, do not focus artificial light perfectly. This error can be overcome in the following manner: Focus the lens upon the object, remove the ocular, look into the tube, and so adjust the mirror that the image of the source of light can be seen in the lens at the bottom of the tube. If the condenser is now gently lowered by means of the substage screw, the image will disappear and the lens become brilliantly illuminated throughout. When the point of brightest illumination is reached, replace the ocular. The microscope field will now be found to be amply illuminated.—Brooks.]

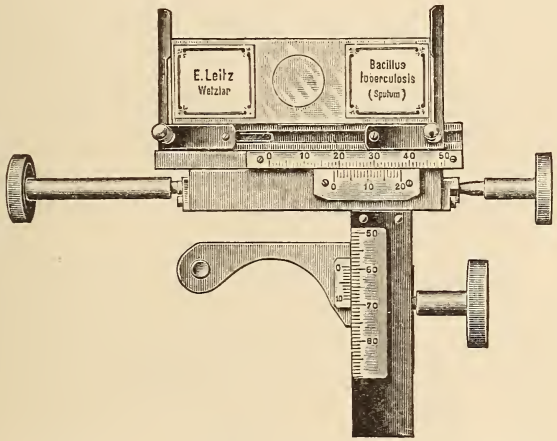


Fig. 16.—Leitz Double-Geared Mechanical Microscope Stage.

With high magnifications, which should, as a rule, be secured with strong objectives, and *not with strong oculars,* a small diaphragm opening is to be employed. The homogeneous immersion lenses will stand very strong oculars. The Abbé condenser need not be removed during observation of tissues, because when a *small diaphragm aperture* is used structural details are preserved. On the other hand, if stained details are to be examined, all obstruction to illumination should be removed or the iris diaphragm opened wide, in order to secure the fullest effect of light. In this way the histologic outline—structure—is almost wholly obscured, while *colored parts* are more distinctly brought to view.

In examining with *high-power dry lenses* it is advisable to employ cover-glasses of a thickness adapted to the lens. In the better makes of Zeiss lenses the metal casing is numbered to indicate the cover-glass thickness for which the lens is most perfectly corrected. Cover-glasses

of medium thickness measure from 0.15 to 0.2 millimeter [$^1/_{150}$ to $^1/_{125}$ inch] upward. With the homogeneous oil-immersion lenses the thickness of the cover-glass is not of such great importance. [In using the microscope it is advisable early to acquire the habit of keeping *both eyes open*.—Brooks.]

The length of the microscope tube [see Fig. 3, *D*] is also a matter for consideration, because the objectives are adjusted for a certain tube-length. The tabulated card accompanying every good microscope indicates the tube-lengths, with which the different magnifications there given are obtained.

It is not rare for the field of the microscope to be obscured by bright reticulated or branching lines and dark and light points. These

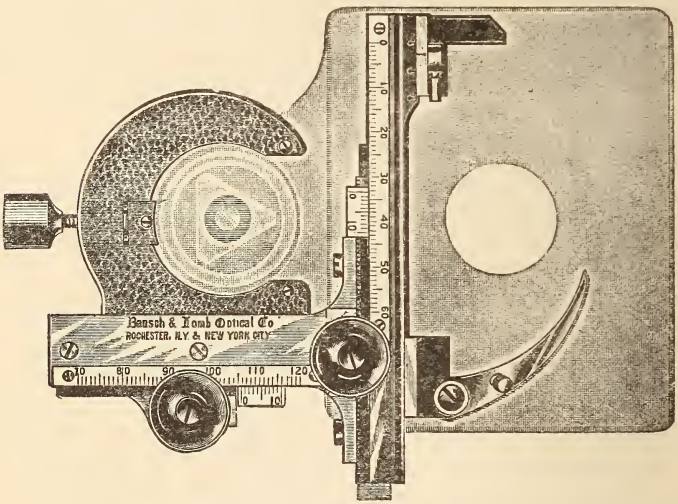


Fig. 17.—Bausch & Lomb Mechanical Stage in Position on Microscope.

are due to entoptic phenomena, which now and then occur even with ordinary vision, and are known as "*muscæ volitantes*." Many of the dark points which here and there disturb the view are due to soiling of the optic media. When such are located upon the ocular they can readily be detected by gently turning the latter in the tube and noting whether they move with it. The same remark is also applicable to the objective. The lenses and eyepieces should always be cleansed by gentle concentric rubbing with a soft cloth, moistened with alcohol or benzin. Not infrequently minute fragments of the cloth with which the lenses or slides are wiped are left upon the glass. With the novice these may lead to errors of interpretation. *Therefore the beginner is strongly*

advised to familiarize himself with the microscopic characters of cotton-, wool-, and silk- fibers, in order to avoid future embarrassment from these sources [see Fig. 18].

[Fig. 18.—Organic Matters frequently Present in Dust. (After *Heitzmann*.)

S, Fibers of silk; *C*, of cotton; *L*, of linen; *W*, of wool. *F*, Feather. *St*, Starch-granules. *Cr*, Cork. *O*, Torulæ. *M*, Mycelia, or threads, of mildew. *Mc*, Micrococci. *B*, Bacteria. *Lt*, Leptothricial filaments. × 500.]

IV. The following *reagents* should be at hand:—

1. Physiologic salt solution (0.7 per cent. of sodium chlorid) as an indifferent fluid, which, like the remaining reagents, is best added to the specimen by allowing it to flow beneath the edge of the cover-glass.

2. Acids:—

Acetic acid, usually in 0.5- to 2-per-cent. solution. It clears up the albuminous substance of the cell-bodies and the connective-tissue fibers, and renders them transparent. The cell-*nuclei* of the elastic fibers, and fat, as well as micro-organisms, are unaffected by it, and are therefore made sharply prominent by contrast with the remaining cleared substances. Mucin is precipitated, and is not dissolved even on addition of an excess of the acid, while fibrin is usually very rapidly cleared up and dissipated.

Hydrochloric and *sulphuric acids* are employed in 0.5-per-cent. solution for decalcifying. In the use of the former CO_2 is liberated; with the other, lime crystals are formed. In 1 to 1000 solution they act like acetic acid. As an addition to alcohol (about 3 per cent.) hydrochloric acid especially is employed as a decoloring agent. This hydrochloric acid-alcohol is permanent.

Osmic acid, in 0.5- to 1-per-cent. solution, is employed for the detection of fat, which is colored black by it, and also as a preservative in the examination of fresh blood, etc.

3. *Potassium* and *sodium hydrate* [caustic] are used in 1- to 3- or at most 5-per-cent. solutions. They gradually clear up or dissolve albumin, connective tissue, and cell-nuclei; on the other hand, lime, pigment, fat, and *elastic tissue*, as well as micro-organisms, are unaffected by them.

4. *Glycerin* should be used in the pure state. By virtue of its great refractive index it serves as an excellent clearing reagent. At the same time it may be employed as a preservative, because it is not subject to evaporation, and it does not form chemic combinations except in the case of fat, which is rendered invisible by it.

5. *Alcohol* is often employed as a hardening (blood) and decoloring agent. *Ether* and *chloroform* are used as reagents upon fat. Alcohol and ether combined [equal parts] serve as hardening reagents. Alcohol combined with 10-per-cent. acetic acid or 3-per-cent. nitric or hydrochloric acid is a *strong* decoloring solution. For ordinary use 1 per cent. of hydrochloric acid in 70-per-cent. alcohol is of service.

6. *Formol* [*formalin, formaldehyd, formalose*], which contains as active constituent 40 per cent. of formaldehyd in a mixture of methyl alcohol and water, is unexcelled for rapid hardening of dry blood preparations (see page 150). In 10-per-cent. watery solution it can be highly recommended for hardening fresh tissues, as it preserves the form, color, transparency, and staining properties.

7. *Staining reagents:* the *anilin dyes* are extensively used as staining reagents. In bacteriologic examinations the basic dyes chiefly are

employed. In the examination of tissue-cells, in addition to the basic dyes, the neutral and acid dyes also are made use of. The methods for their employment will be given in the sections devoted to the examination of bacteria and of the blood.

In addition to the anilin dyes, *iodin* and *hematoxylin* are not infrequently employed. Watery solutions of *iodin* stain the albuminates and connective-tissue substances a pale-yellow color, and render the nuclei prominent. The red blood-corpuscles assume a brownish tinge, and the so-called corpora amylacea a wine-red or dark-brown color. It is best employed in varying dilutions of Lugol's solution (iodin, 1; potassium iodid, 2; distilled water, 100). Preparations treated with it are not permanent, because the iodin is readily discharged. A saturated solution of gum may be recommended as an imbedding agent.

Hematoxylin is an excellent nuclear stain. Dissolved in alcohol it has a brownish color, which is changed to a blue on addition of alum. The blue solution is used for staining purposes. By combination with *eosin,* which is chiefly a protoplasmic stain, beautiful double staining can be secured. Detailed description of the composition and method of employing these solutions will be given in the section on the examination of the blood.

8. A few drops of an alcoholic *solution of sudan III* imparts to intracellular fat a bright-red color.

9. *Canada balsam,* dissolved in either pure xylol or chloroform, is employed for imbedding preparations. *Balsam copaiba* and *cedar-oil* are also adapted for the same purpose. The transparency of the preparations is enhanced by the use of these balsams.

V. Necessary or desirable accessory *apparatus* are as follows: 1 or 2 *anatomic forceps,* with delicate branches; 1 *Cornet forceps,* which is adapted especially for staining dry cover-glass preparations; 1 small *scissors;* 1 small *scalpel;* 2 preparation needles; 1 platinum-wire inoculator; slides and cover-glasses; 1 alcohol-lamp [or Bunsen burner]; several small glass rods; pipettes, assorted sizes; test-tubes; several watch-glass dishes; 2 glass funnels; several conic sediment glasses; 1 or 2 porcelain dishes; filter-paper, and labels.

I. THE VEGETABLE AND ANIMAL PARASITES.

(A) VEGETABLE PARASITES.

The present known exciters of the infectious diseases *belong to the lower fungi.* As their systematic division has been subject to much change, it appears to us advisable to adopt the classification of Flügge-Frosch, which is as follows: *1. Fission fungi, schizo- (schisto-) mycetes, or bacteria. 2. Streptothrixiæ. 3. Budding fungi, blastomycetes, or yeasts. 4. Thread fungi, hyphomycetes, or molds.*

1. BACTERIA.

General Preliminary Remarks.

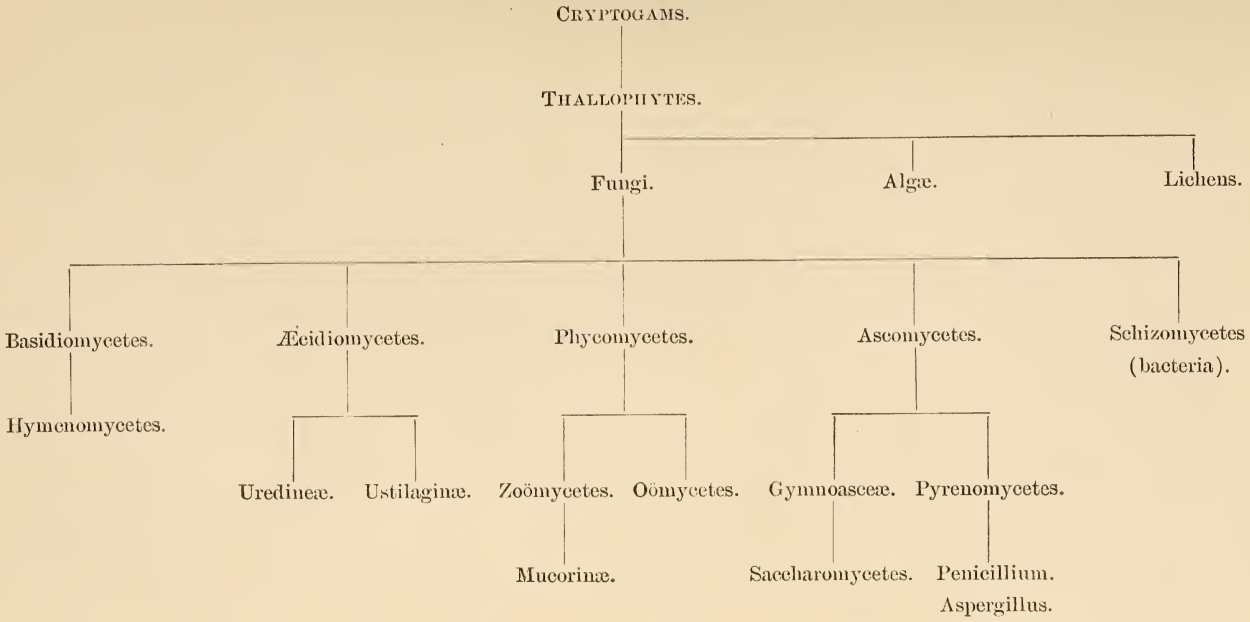
Since the fundamental investigations of Ferdinand Cohn and others, the bacteria in general have been placed in the vegetable kingdom, as their elementary forms grow and divide like vegetable cells. According to Naegeli and others, they are also designated as *fission fungi* (schisto- or schizo- mycetes), because, like the fungi, they are devoid of chlorophyl.

The single cells of the bacteria consist of a protoplasmic substance, without a nucleus, inclosed within a cellulose or albuminous envelope. The latter plays an important part in the division of the cell as well as in the formation of cell-conglomerations (zoöglœa); it may swell by taking up water and change into a gelatinous state.

In the absence of more accurate differential characteristics the bacteria are classified according to their morphologic appearances as: *(a)* spheric bacteria, or *micrococci; (b)* rod-shaped cells, or *bacilli;* and *(c)* corkscrew-shaped organisms, or *spirilla.* With but few exceptions the first never exhibit true independent motion, while with a great number of bacilli and all spirilla more or less independent motility is observed.

Independent motility is always produced by very delicate *flagellate threads,* which are usually attached to the poles of the

THE POSITION OF BACTERIA IN THE VEGETABLE KINGDOM. (AFTER EYRE.)



organism; at times but *one* polar flagellum is present; at others, a whole bunch of them may be seen. Finally, many bacteria appear surrounded by erect flagella. [See Fig. 19.]

According to Fischer, this diversity in the distribution of the flagella might serve as a means of classification. Among the bacilli he

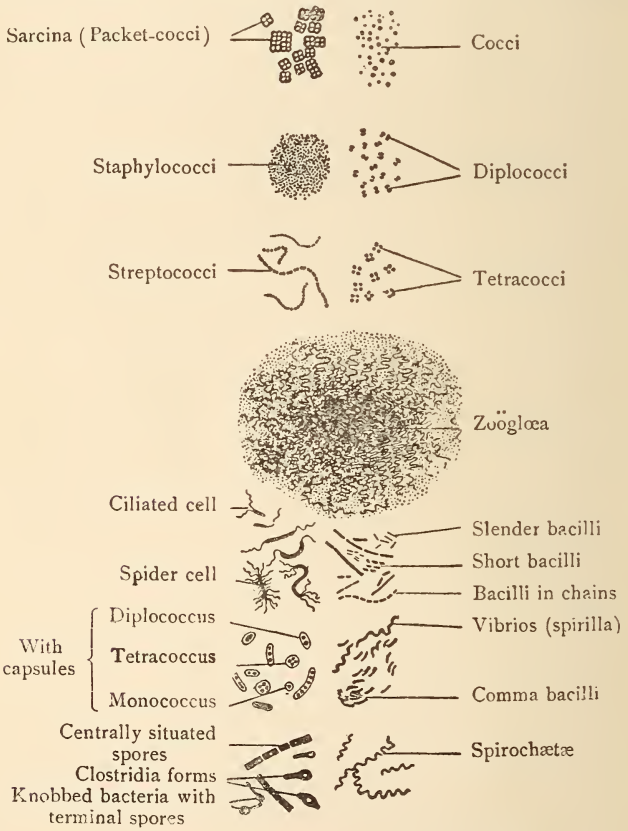


Fig. 19.—Forms of Bacteria. (From *Schenk.*)

distinguishes: 1. Such without flagella: *bacilli.* 2. With *one* polar flagellum: *bactrinia.* 3. With a bunch or tuft of flagella: *bactrilli.* 4. Diffusely covered with flagella: *bactridia.*

The detection of the flagella is not always easy. The methods employed for staining the flagella will be spoken of when discussing the typhoid bacillus (see page 61).

The bacteria are propagated either by *fission* or *spore-formation.* In the first process the cell is divided into two—almost equal—parts by a transverse fissure starting from its envelope; or division takes place not only in one, but in two or all three directions of space. Accordingly we meet with bacteria dividing into pairs (diplococci), or those which lie in fours (tetracocci), or the sarcina (packet-cocci) formation. If the diplococci remain united in long rows they are spoken of as *streptococci* (chain-cocci); if they appear more in the form of bunches they are designated as *staphylococci* (clumped cocci).

Spore-formation occurs (perhaps) in two different ways: in the one, the so-called *endogenous* spore-formation,—which has been positively proved and first studied with precision upon the anthrax bacilli,—a stronger refractive zone forms within the mother-cell, developing more or less rapidly into a round, usually more ovoid, spore, which appears to be surrounded by the remaining light portion of the mother-cell. With

[Fig. 20.—Forms of Bacilli showing Spores. (From Oertel, courtesy of P. Blakiston, Son & Co.)]

complete ripening of the spore the outer membranes give way and the spore becomes free. Under favorable conditions it then begins to germinate, appears less refractive, becomes more and more elongated, and finally resembles the mother-cell in all particulars.

According to some investigators, spore-formation begins only after exhaustion of the nutrient medium: *i.e.,* when the preservation of the species is endangered. However, it has positively been determined that *a supply of oxygen is absolutely necessary for their development, and that certain limits of temperature must not be exceeded.* The second form of sporulation is that designated as *arthro,* or *joint spore-formation.* In this form of sporulation certain portions of the cells—which are by no means morphologically well characterized—separate by constriction and form a resting or "lasting" [*Dauer*] spore. Further researches have much to contribute to the solution of this problem.

The spores present *true permanent forms.* They are distinguished from the mother-cells by their pronounced powers of resistance. In contrast to the mother-cells, they are also

distinguished by the fact that they take up staining reagents only under certain conditions later to be described in detail. In ordinary staining they appear in the tinged protoplasm of the mother-cell as bright, uncolored gaps.

Owing to this circumstance, the rods possessing such colorless zones were formerly called "spore-containing" bacilli (*e.g.*, in the tubercle bacillus). Therefore it may here be emphasized that such light-zones may appear as a sign of degeneration as well as a result of "preparation *plasmolysis.*"[1] In certain instances a decision in this regard is not easy. Strictly speaking, observation of germination is the only proof of endogenous sporulation.

The plasmolytic processes occurring during treatment of the bacteria with staining reagents, which have been studied particularly by botanists, notably A. Fischer, deserve our closest attention.

Temperatures under 5° and over 50° C. [41° to 122° F.] may be regarded as the limit for the life and growth of the bacteria. The so-called *pathogenic* bacteria, which are recognized as the exciters of the infectious diseases, *thrive best at the temperature of the body,* while the nonpathogenic grow best at a much lower temperature—about 20° C. [68° F.]. Fermentation and putrefaction, as well as the formation of pigments and acids, are to be considered among some of the effects of this group.

According as a *supply of oxygen* is necessary, detrimental, or indifferent for the growth of the bacteria we distinguish *obligate aërobic, anaërobic,* and *facultative anaërobic* bacteria, respectively. To the latter group belong almost all pathogenic microbes.

Those bacteria which are capable of developing only within the living animal body are designated as *strictly (obligate) parasitic bacteria,* and those which are capable of living and multi-

[1] By addition of $\frac{1}{10}$-per-cent. salt solutions, which are allowed to act from the margin of the cover-glass, brightly glistening bodies may be produced within originally homogeneous fungous threads. On washing with water these bodies disappear. To all appearances they are originated by separation of the protoplasm from the cell-membrane and its contraction into clumps ; after washing out the salt solution the protoplasm expands to its former dimensions. Varying with the length of the bacteria, we sometimes observe one, sometimes two, or even a number of bright zones which the inexperienced observer may easily mistake for spores. Their occurrence on addition of salt solution and their disappearance after washing readily convince one that they are artefacts.

plying only upon dead organic material are termed *saprophytic bacteria. Facultative parasites* and *facultative saprophytes* are terms used to designate such bacteria which, although primarily depending upon one or other of the soils just mentioned, preserve the power of development upon both.

The metabolic products of the bacteria set a limit to their multiplication and activity. Unfavorable soil gives rise to bad growth and degeneration forms.

A species of bacteria may be regarded as *specifically pathogenic* only when it can be microscopically demonstrated in *all* cases of a certain disease, and in it *exclusively,* and when inoculation of "pure cultures" of the species into other bodies always produces the same disease (Koch).

Not all of the bacteria to which we are inclined to attribute a specifically pathogenic rôle have wholly fulfilled the requirements of the above-mentioned postulate. This is due to the fact that the methods originated and brought to such a degree of perfection, especially by Koch and his pupils, are as yet incomplete; furthermore, with many species of bacteria animal experimentation gives negative results, because these bacteria find only within the human body their true soil and the conditions essential for growth and the development of their specific pathogenic effects.

GENERAL REMARKS UPON THE EXAMINATION OF BACTERIA.

Demonstration of Bacteria by Cultivation Methods.

It would lead us beyond the limits of the present treatise to discuss the culture methods of R. Koch and his school at such length as to enable the beginner to work after the directions that would be given. For this purpose reference may be made to the excellent text-books on bacteriology by Flügge, C. Fränkel, Günther [McFarland, Novy, Park, Muir and Ritchie, Eyre], and others. The procedures for cultivation may, however, be so sketched that the beginner can at least gain some idea of the fundamental principles, etc. To the imperishable researches of R. Koch we owe our knowledge of the isolated cultivation of bacteria upon solid and transparent nutrient media—the "**pure culture.**"

In examining material containing bacteria we must not, for obvious reasons, lose sight of the fact that, in addition to the true pathogenic bacteria, a greater or lesser number of other species is usually present.[1] First of all, then, our efforts should be directed toward securing *separated growths* of the different bacteria. This can be accomplished by *diluting* the material to be examined as much as possible; distributing it in a liquid, coagulable nutrient medium, and spreading the latter upon a "plate" in such a manner that the colonies formed by the different kinds of germs may develop widely separated from each other in a fixed position. In the method soon to be considered more in detail a clouding of the "plate" [better in a Petri dish] can usually be noticed with the naked eye after twenty-four hours, often sooner.

With the aid of hand-glass or weak objective this cloudiness is recognized as due to isolated colonies. From the appearance of the colonies, from the presence or absence of "liquefaction of the nutrient medium," etc., we have definite points of distinction which invite to more accurate study of the species in question. To this end a distinctly isolated colony [or a portion of it] is taken ("fished") up with a heated (and subsequently cooled) platinum needle,[2] the hand being carefully guided by a lens or microscope, and transferred to a test-tube containing gelatin or other nutrient medium. Provided no technical error has been made, but *one* kind of bacteria must develop here. (The "fishing" requires much practice!)

We distinguish *solid* and *liquid nutrient media*, and, under the former, again, such as resist the temperature of the incubator,[3] and such as remain solid at low temperature, but liquefy at a somewhat higher one. Since the growth of the bacteria is, in great measure, dependent upon the degree of temperature, it is of the utmost importance that we should have at our disposal the various kinds of media. Moreover, as the form of the colonies upon each particular medium is more or less characteristic, we are able to cultivate the same species of bacteria on several media at the same time and to make use of the different appearances of growths as a means of recognition.

Of the *solid nutrient media* suitable for culture at *low* temperature (below 25° C. [76° F.]) *nutrient gelatin* is the most important; it is used for "*plate*" [Petri dish] and "*stab culture*." It is prepared from an infusion of meat, to which common salt, pepton, gelatin, and pure soda

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[1] For exceptions see, among others, cholera, diphtheria, etc.

[2] [It is advisable, also, to heat the lower half of the glass rod which holds the platinum needle.—BROOKS.]

[3] The desired degree of temperature is obtained in the so-called "brooding oven" (thermostat or incubator), a double-walled copper chamber covered upon the outside with felt [or asbestus (see Fig. 21)]. It usually contains two compartments, each of which can be closed by a thick glass door and a copper-felt door. Between the walls of the chamber is warm water, the temperature of which can be read off from without by means of a thermometer. The degree of heat is regulated by an especially constructed apparatus (thermoregulator), the gas-supply being controlled by mercury or colored alcohol as soon as a certain temperature is reached.

are added. The method of *preparation* is as follows: 500 grams [1 pound] of finely ground beef [see Fig. 22], free of fat, are carefully stirred up with 1 liter [1 quart] of distilled water. After standing for

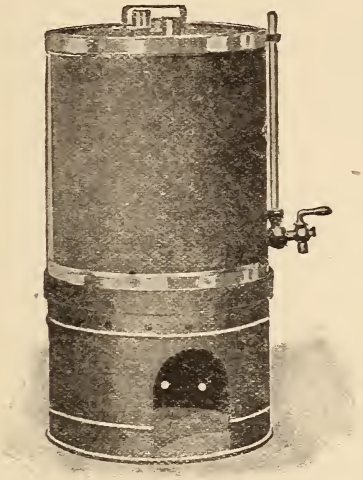


Fig. 21.—Bausch & Lomb Physician's Incubator.

twenty-four hours in a cool place the infusion is strained and gently squeezed through a cloth; so that in all about 1 liter [1 quart] of meat-juice is obtained. [If the full amount is not obtained, sufficient water may be added to raise it to 1000 grams (1 liter).—Brooks.] To this are added

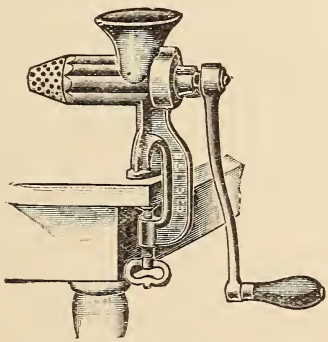


Fig. 22.—Meat-grinder.

10 grams [2 1/2 drams] of pepton (siccum), 5 grams [1 1/4 drams] of common salt, and 100 grams [3 1/4 ounces] of commercial gelatin. This so-called "nutrient bouillon" is now allowed to swell and subsequently

to dissolve in a water-bath. This is followed by addition of pure soda [sodium hydrate or bicarbonate] in saturated watery solution until a distinct alkaline reaction (with litmus-paper) is just visible.

By heating for two hours in a steam sterilizer [see Figs. 23 and 24] the coagulable albumin is precipitated. On careful filtration of the hot liquid [through paper or absorbent cotton] a *perfectly clear* and *transparent mass* is obtained, which must still give a distinct alkaline reaction. [Filtration may be facilitated by the use of the hot-water funnel[1] (see Fig. 26) or by placing the flask and funnel in the steam sterilizer (Fig. 23).—Brooks.] The clear filtrate can now be poured, in amounts of 10 cubic centimeters [2 1/2 drams] each, into test-tubes, previously sterilized [in an oven, Fig. 25], which should be closed with tightly fitting cotton plugs before and immediately after filling [see Figs. 27 and 28].

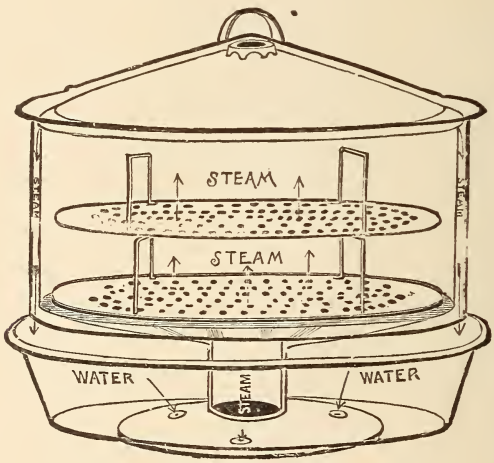


Fig. 23.—Arnold Steam Sterilizer.

Finally the filled test-tubes are placed in the steam sterilizer, where they are heated at the boiling-point for twenty minutes. This process is repeated once a day on three or four successive days in order to kill all germs at first present, as well as to destroy such bacteria as may develop anew from spores in the intervals of heating.

The *nutrient gelatin* thus prepared is first employed for "*plate culture.*" By carefully heating the lower portion of a gelatin tube the contents are liquefied.[2] A minute portion of the material containing the bacteria is now taken up by means of a platinum loop (previously heated to redness, and subsequently cooled) and transferred to the liquid

[1] [Usually one-half hour suffices.]

[2] [It is best to liquefy the gelatin in a water-bath at a temperature of about 40° C.—Brooks.]

gelatin in the tube. Since a widely separated (isolated) growth of the bacteria is required to secure a pure culture, it is, in the majority of instances, necessary to further attenuate the mixture of organisms and gelatin. This may be accomplished by transferring two or three

Fig. 24.—Autoclave.

platinum loopfuls from the first inoculated tube of liquefied gelatin to the second, and two or three loopfuls from the second inoculated tube to a third tube of liquefied gelatin. During this procedure care must be observed that the glass tubes are opened but for an instant, and that

the platinum loop is heated to redness and again cooled before and after each inoculation from tube to tube [see foot-note on page 20]. Especial attention must be directed to the cotton plug, as upon its sterility depends the success of the pure culture. Only that portion of the plug which is outside of the tube should be touched with the fingers [and no portion of it, except that touched by the fingers should come in contact with any object—BROOKS]. When making the inoculation, it is best to hold the plug in the left hand—the hand which also holds the tube.

The inoculated tubes are now ready to be "poured upon the [sterilized] plates." The so-called Petri double dishes serve as plates, the upper one of which serves as a complete cover for the lower. Before

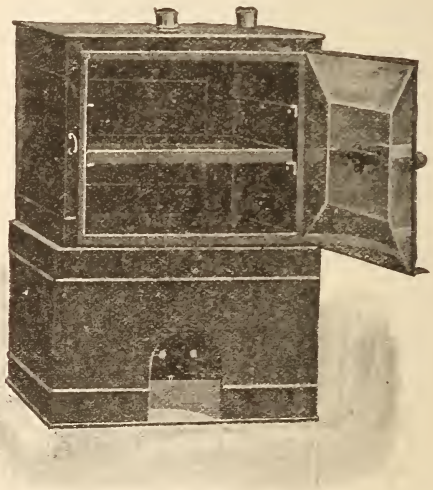


Fig. 25.—Hot-Air Sterilizer.

"pouring the plates" it is advisable carefully to heat the mouth of a tube over a flame, after the cotton plug has been removed, in order to kill any germs which may be present within it. Then the cover of the Petri plate is quickly raised, the medium poured into the lower dish, and the cover at once replaced. [See Fig. 25*a*.]

The plates are now set aside at ordinary room temperature (17° to 18° C. [62° to 64° F.]) for twenty-four hours, after which time development of the colonies can frequently be observed [see Fig. 29]. From the "pure cultures" which develop here (the isolated position of the colony must be confirmed by a hand-lens or a low-power objective) one of the colonies is taken up with the platinum loop for *further culture in tubes*. The solid gelatin contained in the tubes is inoculated by

stabbing with the platinum wire which carries a bit of the pure culture ("stab culture" [Fig. 30]). The tube to be inoculated is held base upward, the cotton plug quickly withdrawn, the stab made, and the cotton plug at once replaced.

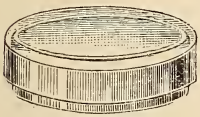


Fig. 25*a*.

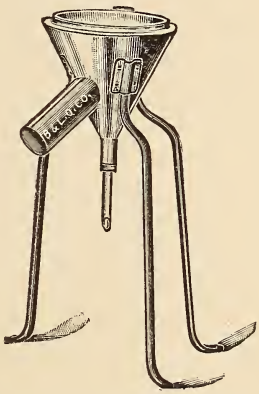


Fig. 26.—Hot-Water Funnel for Filtering Agar and Gelatin.

Of solid media adapted for *cultivation in the incubator, nutrient agar-agar, blood-serum,* and *potato* may be mentioned.

Nutrient agar-agar is prepared by adding (instead of gelatin) 10 to 20 grams (0.5 per cent.) of agar-agar to "nutrient bouillon" which has previously been boiled for about one hour in the steam sterilizer and freed from albumin by filtration (see above). This mixture is boiled

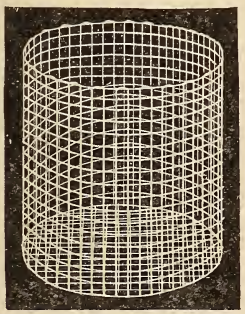


Fig. 27.—Wire Basket for Test-tubes.

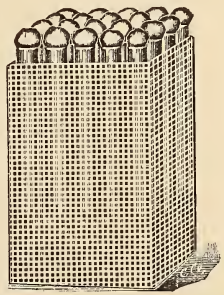


Fig. 28.—Wire Basket for Test-tubes.

until the agar-agar is completely dissolved, when sodium hydrate or bicarbonate is added up to the point of faintly alkaline reaction [as shown by blue coloration of red litmus-paper—Brooks]. After boiling again for several hours the solution is carefully filtered [while hot, by aid

of hot-water funnel, through a filter-paper or absorbent cotton, moistened with boiling water—Brooks]. The process of filtration is very tedious. The liquid nutrient agar-agar is poured into test-tubes [and sterilized as directed for "gelatin"—Brooks]. In order to obtain a large surface upon which the germs may develop, the agar in the tubes is allowed to solidify in a slanting position [Fig. 31]. During solidification of the agar, so-called "condensation water" is pressed out and collects at the bottom of the tube.

Blood-serum is obtained either from the human placenta or from the freshly opened blood-vessel of an animal. Some of the first blood to flow is allowed to escape, in order to wash off any germs which might be adhering to the skin and hairs. After the serum has separated (in a cool place) it is drawn off and poured into sterile test-tubes, where it is allowed to coagulate at 68° C. [154.4° F.] in a slanting position (as in agar-agar). To test its sterility the tubes are kept for three or four days at incubation temperature—about 36° to 37° C. [97° to 98.6° F.]. If they remain absolutely free from germs they can be used with safety. [Hydrocele or ascitic fluid may be employed in the same way.—Brooks.]

Potatoes are used either in the form of simple halves or sliced. In either case the potato is thoroughly scrubbed under the water-faucet to free it from all dirt, and the eyes and any decayed parts are carefully cut out with a knife. If both halves are desired for culture purposes, the *sound* skin is left intact as far as possible, and the potato placed for one hour in a 1 to 1000 solution of mercury bichlorid. The potato is then cooked for from one-half to three-fourths of an hour in the steam sterilizer, after which it is cut into halves with a heated (and subsequently cooled) knife, the hand holding the potato having previously been thoroughly cleansed with sublimate solution. If possible, the potato is inoculated at some distance from its edge (one centimeter [about one-half inch]); it is then placed in the "moist chamber," consisting of two glass dishes, one fitting over the other, the bottom of which is covered with a layer of moistened filter-paper.

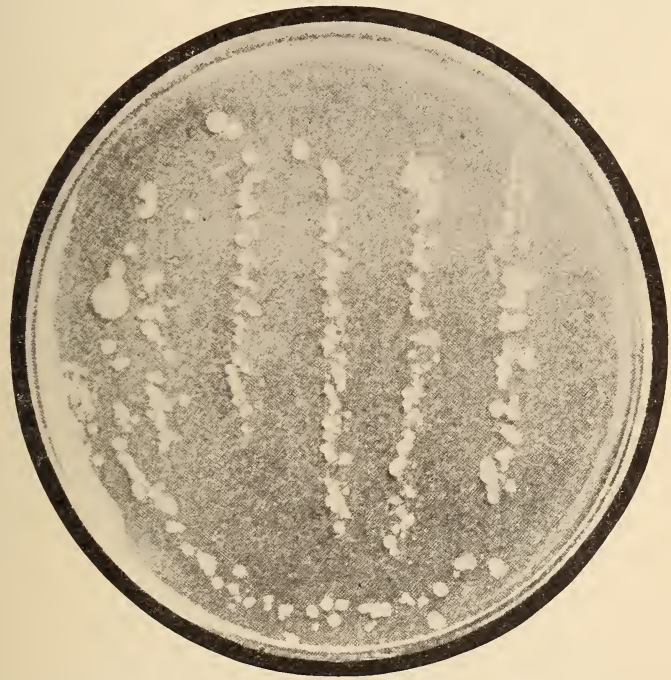
The method of *Esmarch* is much simpler. The well-cleansed potato is peeled and sliced into disks about one centimeter [one-half inch] thick. One of these disks is placed in each of a number of sterile double (Petri) dishes and steamed for from one-half to three-fourths of an hour in the steam sterilizer. By this procedure the disks are cooked and the plates sufficiently sterilized at the same time.

[*Bolton's* method is still more simple. In this method a number of cylindric pieces of potato, about one-half inch thick and one and one-half inches long, are first prepared and then cut diagonally so as to give a large surface. One piece is placed in each test-tube along with a few drops of water, and the tube is plugged with cotton and then sterilized as in ordinary potato media.—Brooks.]

To prepare the culture, the material containing the bacteria is *lightly* smeared over or rubbed into the disks of potato or upon the gelatin or serum medium.

The form, color, and density of the growths upon potato cultures are often very characteristic; upon the other media also the appearance of the culture is not infrequently of decisive character.

Pure cultivation of the *anaërobic* bacteria succeeds only when oxygen is excluded. In plate cultures oxygen can be excluded by laying a small sterilized mica plate upon the medium. In vessels the oxygen must be displaced by the introduction of pure hydrogen, and the opening hermetically sealed with paraffin. According to C. Fränkel, the hydrogen



[Fig. 29.—Plate Culture. (From Oertel, courtesy of P. Blakiston, Son & Co.)]

prepared from pure zinc and pure hydrochloric acid requires to be freed of sulphureted and arseniureted hydrogen and traces of oxygen. For this purpose the hydrogen generated is passed through three wash-bottles which contain alkaline lead solution, silver nitrate solution, and alkaline pyrogallous solution, respectively.

The Microscopic Examination of Bacteria is always necessary, and will, therefore, be fully considered. It is carried out with *stained* and *unstained* preparations.

1. The Unstained Preparations are examined either by placing upon a glass slide a small portion or drop of the material, gently pressing upon it a cover-glass, and viewing the whole preparation with a low- and a high- power lens, or the observation is made in the *"hanging drop."*

The first method will very rarely suffice, because too many difficulties attend its use. Differentiation of the bacteria is indistinct; observation is interfered with by the active phenomena of motility, which are due partly to independent motion or—

[Fig. 30.—Stab Culture. (From Oertel, courtesy of P. Blakiston, Son & Co.)]

[Fig. 31.—Smear Culture. (From Oertel, courtesy of P. Blakiston, Son & Co.)]

as is always the case with cocci—to *Brownian* molecular motion and fluid currents. An oil-immersion lens and an Abbé illuminating apparatus (condenser) are best adapted for examination, but an iris diaphragm must be inserted, since, otherwise, the structural details are entirely obscured by the intense illumination.

2. Examination of the "Hanging Drop" is of utmost importance. It not only enlightens us as to the form, but also as to the manifestations of life (motility) of the bacteria [Fig. 32].

Directions.—Moisten a scrupulously cleansed cover-glass with a drop of the fluid to be examined and place over this a hollow-ground glass slide—the edges of whose central depression is smeared with vaselin—in such a manner that the drop projects exactly into the cavity of the slide; if a piece of tissue or a culture is to be examined, a trace of the material is added to a drop of fresh water or sterile [so called] physiologic salt solution [0.6 to 0.75 per cent.] and then carefully rubbed upon the cover-glass until perfect distribution is secured. The preparation is then inverted and examined in the usual way—best done with an oil-immersion lens and Abbé condenser, but with a nearly closed iris diaphragm, since the objects to be examined are unstained. It is best first to examine the marginal portions, because the morphologic and biologic characters of the bacteria are more easily studied in as thin layers as possible. For the sake of simplicity a low-power lens is used first; the latter is replaced by the oil-immersion lens after the marginal zone has been brought into the field of vision.

This method is almost exclusively employed in the examination of pure cultures. The advantages of this method over those first mentioned are that with it *the natural form and movements of the bacteria can be seen, and that observation can be continued for hours at a time, since evaporation is almost wholly excluded.*

Fig. 32.—Hanging-Drop Preparation.

By this means alone, however, the present state of bacteriologic knowledge could not have been attained. The accomplishment of this end required the development of the *methods of staining* now almost universally adopted. Here, also, R. Koch deserves the distinction of having conceived and employed the fundamental methods. Next to him, Ehrlich, Weigert, Baumgarten, and the numerous pupils of Koch and others have contributed greatly to the modification and perfection of staining technique. Of decided importance in the elements of success were: The introduction of the oil-immersion lens in conjunction with the Abbé illuminating apparatus, *the adoption of an appropriate procedure for securing "cover-glass dry preparations," and the application of the anilin dyes.*

Method of Preparing Cover-glass Specimens.

With the aid of a previously sterilized steel needle or platinum loop a very small portion or drop of the material to be examined is placed upon a carefully cleansed cover-glass and so covered with a second cover-glass that the edge of the one projects slightly beyond the other. If the slight pressure exerted by the cover-glass does not serve to spread the object into a thin film, a gentle touch with a needle will suffice. The cover-glasses are then quickly, but gently, slid (not lifted!)

apart by the aid of two forceps. After some practice the glasses may be prepared by seizing the two projecting edges with the fingers and then sliding them apart. All handling of the cover-glass surface must be avoided.

An experienced worker can get along with but *one* cover-slip [or slide] by lightly smearing a small quantity of the material to be examined over the surface of the glass by means of a platinum loop, glass rod, or steel needle. With fluids it is advisable to place a small drop near one edge of the cover-slip and spread it quickly, but gently, over the surface of the latter with the edge of a polished object-glass, larger cover-glass, or other object.

1. If a young culture is to be examined, the cover-glass is gently pressed upon a colony on the Petri dish and at once lifted away. It is then treated as directed below under Nos. 2 and 3 ("this is called an impression preparation"). The form of the bacteria as well as their position in the colony can thus be recognized.

2. The preparations are set aside, smeared side up, *until thoroughly dried by the air.* The process of drying can be hastened by passing the preparation back and forth at some distance (about one-half meter [nineteen inches]) above the flame, or simply through the air.

3. It is now necessary to *transform the albuminous substances of the preparation into an insoluble state.* This is done by *heating.* The simplest plan is that recommended by Koch, which consists in *passing the thoroughly dried preparation (smeared side up) three times through the flame.* In this way a permanently "fixed" preparation is obtained which can be treated for hours or days with staining solutions without injury. In the absence of such treatment the structures are generally blurred, as a result of solution and swelling of the albuminous and mucous substances.

Beginners frequently make the mistake of fixing over the flame before the specimen is completely dried; an obscure picture is the result, and impatience is punished by loss of time. Furthermore the preparation must not be heated too strongly. Therefore the habit of passing the specimen three times through the flame must be acquired early. Only the fixation of dried blood-specimens requires a greater number of transits through the flame—at least five to ten times—or heating above a flame for one or two minutes. A method still more to be recommended is the fixation of such preparations in alcohol or formalin. The thoroughly air-dried preparation is placed for from fifteen to thirty minutes in absolute alcohol; or in a mixture of absolute alcohol and anhydrous ether, equal parts; or in a solution of formalin (see "Blood").

Aside from fixation of the albuminous bodies, which also resist treatment with staining solutions for hours, we obtain by this procedure complete quiescence of the bacteria, which greatly

facilitates a thorough study of the morphologic characters. [The slide method is much more convenient and practical. See page 32.—Brooks.]

Staining of Dry Cover-glass Preparations.

To demonstrate the bacteria in the dry cover-glass [or slide] specimens prepared in the manner described, the smeared surfaces are treated with solutions of the anilin dyes—substances obtained from coal-tar during the manufacture of illuminating gas and distinguished for their great affinity for the bacteria.

We distinguish (with Ehrlich) *basic* and *acid anilin dyes.* While the former are chiefly to be considered as nuclear and bacterial stains, the latter possess the property of staining cell-protoplasm, and especially the bodies of the red blood-corpuscles. This subject will be referred to in the section upon the blood. In the present instance we have to deal solely with the basic—nuclear—staining substances. These are used in watery and alcoholic solutions. Those most frequently employed are gentian-violet, fuchsin, methylene blue, and Bismarck brown (vesuvin). While the former two stain very intensely and readily *over*-stain, the latter stain more weakly and do not overstain. It is desirable to keep in stock a concentrated *alcoholic* solution of the first two dyes mentioned, while of the last two mentioned a concentrated *watery* solution may be kept on hand or a fresh solution made as needed.

Staining.—About 5 or 6 drops of a concentrated alcoholic solution of gentian-violet or fuchsin are added to a watchglassful of water. The dry preparation is allowed to float, smeared side down, upon this mixture, which may be accomplished by seizing the cover-glass at its opposite edges with a pair of forceps and letting it fall flat upon the surface of the fluid from a height of about one or two centimeters [one-half to three-fourths inch].

After a certain time [see below] the cover-glass is removed from the watch-glass with forceps, the excess of stain poured off, and the cover-glass then rinsed in water. During this procedure the cover-glass should be grasped by the opposite edges with the forceps. The specimen is now allowed to dry in the air—which can be hastened by blotting with filter-paper—and imbedded in xylol-Canada or pure copaiba balsam.

A simpler method is to grasp the preparation with a Cornet forceps and pour upon the prepared side of the glass a few drops of the stain, which may be allowed to act as long as desired. To avoid deposition

of disturbing precipitates of the dye, the staining solution may be filtered through paper.

Finally, the staining may be done upon the *object-glass*. The material to be examined is so distributed between two slides that at least one-fourth of the length and one-third of the width of the slide remain uncovered. The air-dried preparation is passed about ten or twelve times through the flame. ["Fixing" is sufficient when the under surface of the glass slightly burns the finger on contact.—Brooks.] The prepared surface is covered with a piece of filter-paper, and this is moistened with the staining solution, added drop by drop. Staining may be done with or without the aid of heat (S'wiatecki). This method offers many advantages. With it we are able to examine a much larger area which nowhere shows a metallic precipitate (as is often the case with the cover-glass method), since the staining solution is filtered. Furthermore the watch-glass is superfluous.

The *time of staining* depends upon the species of bacteria and the strength of the staining solution. This point will be discussed when describing the individual bacteria. Here, however, it may be stated that, the stronger the staining solution, the shorter the time of staining should be, and that *it is generally advisable not to employ too strongly concentrated solutions because of the liability to overstain*. This defect can be remedied by decoloring agents, but many bacteria and nuclei are almost decolored.

Staining of spores requires especial precautions. As a rule, spores take up the dyes only when treated for a long time with intensely staining and heated solutions (see "Anthrax").

[**Fixing Films of Discharges, etc.**—1. Place the slide or cover-glass, film side down, while still wet, upon a saturated solution of bichlorid of mercury in 0.75-per-cent. sodium chlorid for five minutes; transfer to 0.75-per-cent. solution of sodium chlorid for half an hour, occasionally shaking gently to remove the mercury. Then pass through successive strengths of methyl alcohol, remaining a few minutes in each. Stain and treat like specimens of tissue sections. Nuclear structure, mitotic figures, etc., are well preserved thereby.

2. Place films while still wet in the following solution for five minutes or longer:—

Absolute alcohol	25 c. cms.
Ether (C. P.)	25 c. cms.
Mercury bichlorid (alcoholic solution, 2 grams to 10 cubic centimeters)	5 minims.

Wash well in water, then stain. The alcohol may be saturated with eosin and this will act as a counterstain.—Brooks.]

The Staining Power of Solutions is Greatly Increased:—

1. By *heating,* the cover-glass being allowed to float upon a hot solution contained in a watch-glass. The solution may be heated in a test-tube and then poured into the watch-crystal, or the latter may be filled with the solution and held over a flame until steam ascends or small bubbles begin to form at the margins.

2. By the *addition of alkalies,* as in the following formulæ of Koch and Löffler:—

Koch's (obsolete) alkaline methylene blue solution:—

Concentrated alcoholic solution of methylene blue	1.0 part.
Distilled water	200.0 parts.
10-per-cent. caustic potash	0.2 part.

Löffler's alkaline methylene blue solution:—

Concentrated alcoholic solution of methylene blue	30 parts.
1 to 10,000 caustic potash solution	100 parts.

3. By *combination with freshly prepared anilin-water* (Ehrlich's gentian-violet- [or fuchsin] anilin-water solution).

Directions.—To a test-tube nearly filled with distilled water add a layer of anilin-oil about 1 to 1.5 centimeters [one-fourth to one-half inch] high and shake *vigorously* for one or two minutes. The mixture is then filtered. The perfectly clear filtrate must not show any trace of free oil upon the surface. Pour some of the filtrate into a watch-glass and add 2 to 4 drops of a concentrated alcoholic solution of fuchsin or gentian-violet. If the alcoholic anilin-water-gentian-violet solution is frequently employed it is advisable to prepare it as follows:—

5 cubic centimeters of pure anilin-oil are vigorously shaken with

95 cubic centimeters of distilled water and then filtered through a moistened filter-paper. To the clear filtrate, upon the surface of which there must be no visible oil-drops, add

11 cubic centimeters of concentrated alcoholic solution of gentian-violet or fuchsin. The well-mixed staining solution is again filtered through a moistened filter, and to the filtrate are added

10 cubic centimeters of absolute alcohol (as a preservative).

This anilin-water-gentian-violet or fuchsin solution retains its staining properties for *about two or three weeks*, and may be used in a cold or hot state for staining almost all pathogenic bacteria. It also resists decoloring agents more than the majority of other solutions.

4. By *addition of 5-per-cent. solution of carbolic acid* (Ziehl).

Formula.—

Fuchsin or gentian-violet	1.0
Alcohol	10.0
Acidi carbolici liquefacti	5.0
Aquæ destillata	ad 100.0

Aside from its *intense staining power*, this *carbol-fuchsin* (or gentian-violet) *solution will keep almost indefinitely.*

[A simpler formula is:—

Concentrated alcoholic solution of fuchsin[1] (or gentian-violet)	10 c. cms.
5-per-cent. watery solution of carbolic acid (crystals)	90 c. cms.

—Brooks.]

Differential Staining of Bacteria.

Since, in addition to the cells and bacteria, these staining solutions also color small elements, such as nuclear *detritus* and mastcell-granules *(q. v.)*, "selective bacterial staining" is not infrequently demanded to avoid confusion. Of the methods so far recommended, that of Gram deserves the preference.

Gram's Method.—The cover-glasses are stained for from one-half to one minute in a freshly prepared (or only a few days old) anilin-water-gentian-violet solution. After absorbing the excess of the dye with blotting paper the glasses are placed in Lugol's solution.

[The Lugol solution used in Gram's method consists of:—

Iodin crystals	1.0
Potassium iodid	2.0
Distilled water	300.0

—Brooks.]

They remain in this solution for one minute, whereupon they turn quite black. The glasses are now rinsed in absolute alcohol until wholly

[1] [In making this solution care should be taken *not to use acid fuchsin*, which does *not* stain the tubercle bacillus.—Brooks.]

decolored [*i.e.*, until no more color comes away]. After complete evaporation of the alcohol, or, what is preferable, after careful washing in water and subsequent drying, the preparation—which still has a faintly gray color—is imbedded in xylol-Canada balsam. The bacteria only are stained, while all other elements are decolored.

In order to render the bacteria more conspicuous it is advisable to stain the cellular element with a contrast-dye, such as Bismarck brown. For this purpose a watery solution of the latter is allowed to act for half a minute.

More distinct pictures are obtained when the nuclear staining is done first. To this end, Günther recommends staining with Friedländer's picrocarmin solution, which is first allowed to act for from one to two minutes, and after thorough washing in water and alcohol is followed by Gram's method.

The picrocarmin solution is prepared by mixing 1 part each of carmin and ammonia with 50 parts of water and adding sufficient saturated watery solution of picric acid until the precipitate can no longer be dissolved by stirring. Addition of a trace of ammonia quickly dissolves the precipitate.

Gram's method is not adapted for staining all kinds of bacteria, since some, like the cells, are decolored.

Gram's stain is taken up by: the bacilli of tuberculosis, leprosy, anthrax, tetanus, and diphtheria, as well as the pneumococcus of Fränkel, the streptococci and staphylococci, and the micrococcus tetragenus; on the other hand, the bacilli of glanders, cholera Asiatica, typhoid, and influenza, as well as the spirillum of recurrent fever, Friedländer's pneumococcus, the gonococcus, and the plague bacillus are decolored by it. It should be emphasized, however, that Gram's stain does not always give unmistakable results with the diphtheria bacillus or the gonococcus, and that especially the diplococcus intracellularis (Weichselbaum) manifests a decided variability.

Specific Bacterial Staining.

This procedure is by far more valuable and decisive than any other method of staining bacteria. Unfortunately, up to the present time but one such is known, namely: that for the staining of the tubercle bacillus group. These bacilli alone mani-

fest such a high degree of resistance toward the decoloring action of acids as tenaciously to retain the stain after complete decoloration of the remaining parts is obtained (see below).

All stained bacterial preparations should be examined, if possible, with Abbé condenser and oil immersion, but without iris diaphragm, because the large volume of light supplied by the Abbé condenser brings out the details of the stained structures. It must, however, be emphatically stated that the examination for tubercle bacilli, as well as for gonococci, can also be carried out with no small degree of reliability by aid of simple dry objectives having a linear magnification of about 250 to 500. Of course, certain details will escape notice—*e.g.,* the arrangement of the gonococci cannot be sharply defined; but the question as to whether either of these species of bacteria is present in the preparation can be determined with the dry lenses above mentioned. [As has already been stated, the use of any lens except an oil immersion (preferably a $^1/_{12}$) when examining for bacteria should be discouraged. When bacteria are present in large numbers the author's statement holds good; but when they are few and isolated, as is frequently the case, they will more often be overlooked than found, even by the expert, if dry lenses of the powers mentioned are employed.—Brooks.]

THE PATHOGENIC BACTERIA.

In describing the pathogenic bacteria and their microscopic characteristics I shall consider only those forms whose rôle as definite exciters of disease has been established or rendered probable. The large number of bacteria which occur in the mouth, stomach, urine, and stools will occasionally be referred to in subsequent pages. The micrococci, bacilli, and spirilla will be discussed in the order named.

I. MICROCOCCI.

1. In the Various Suppurations.

The **staphylococcus pyogenes** (aureus and albus, Plate VII, Fig. 1) is the exciter of circumscribed suppuration (furuncle, panaritium, tonsillar abscess, empyema, suppurative parotiditis) and generally appears in *conglomerations*. It was accurately described by Ogston, in 1880, and because of the peculiar grouping of the individual cocci was designated as staphylococcus (σταφυλὴ = bunch of grapes). Division takes place in a manner similar to that of the gonococci, but the line of fission is very delicate. Each of the two segments measures on an average 2.1 μ,[1] the individual groups between 3.5 and 10 μ.

Staining.—It is readily and *intensely stained* by all basic anilin dyes, as well as by Gram's method.

It can be cultivated at ordinary room temperature, but grows more luxuriantly at about 37° C. On gelatin plates the colonies appear as delicate white spots, in the neighborhood of which liquefaction begins. A distinct orange color soon develops (hence staphylococcus *aureus*); the color[2] is much more beautifully shown in colonies grown at room temperature upon agar (best in slant culture). In [gelatin] stab culture, also, in addition to active liquefaction, the formation of a golden-yellow colored sediment is characteristic. A species of staphylococcus which liquefies the gelatin more slowly and presents a *lemon-yellow* color is called *staphylococcus pyogenes citreus.*

The staphylococcus possesses very great powers of resistance. It has been demonstrated in the air, sewage, and soil, and, therefore, belongs to the facultative parasites. When it is remembered that the staphylococcus is regularly and exclusively found in a number of suppurations, especially in *acute osteomyelitis* and the general sepsis associated therewith, from which sources it has been cultivated in characteristic pure culture, and that subcutaneous injection—indeed, even rubbing it into the healthy skin—has provoked circumscribed abscesses and furunculous suppurations (Garré), its specific nature cannot be questioned.

It has frequently been found in the *blood*, and in several forms of endocarditis and general sepsis it is the sole etiologic factor.

By injection of the staphylococcus, even in the absence of pre-existing lesions of the valves Ribbert succeeded in producing endocarditic

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[1] μ = micromillimeter = $\frac{1}{1000}$ millimeter [= $\frac{1}{25400}$ inch].

[2] [The staphylococcus albus (white) produces no color.—Brooks.]

processes in animals. Sahli found staphylococcus pyogenes citreus in uncomplicated acute articular rheumatism, in pericarditic and pleuritic exudates, in endocarditic deposits, and in nonsuppurative arthritic effusions. This observation lacks confirmation, as do also those of Wassermann and Litten, who found a peculiar streptococcus in the blood and organs of human subjects dead of rheumatic (?) polyarthritis.

The **streptococcus pyogenes** (Plate VII, Fig. 1) produces erysipelas and the more disseminated phlegmonous suppurations, and for this reason, probably, it gives rise to *general infection* more frequently than the staphylococcus. By a rosarylike arrangement of the individual segments more or less long *chains* are formed, which appear to be composed of pairs united in a row. The size of the cocci often varies. In a case of pneumonia with streptococci the author found that each pair of cocci measured between 1.2 and 1.75 μ. It is easily stained in a few minutes with all basic anilin dyes and also by Gram's method.

The colonies of the streptococcus develop much more slowly than those of the staphylococcus. They do not liquefy the gelatin. Upon gelatin plates the colonies appear as small white dots, attaining at most the size of a pin's head. In stab culture they grow along the needle-track as delicate pearls, arranged in rows, but separated from each other. Upon the surface of agar the cultures appear in the form of delicate, transparent drops. On potato no growth takes place. Cultivation in nutrient bouillon is highly to be recommended; here the growth usually appears as a cloudy sediment and attains a luxuriant development, the supernatant bouillon remaining clear. This by no means constant peculiarity is an important mark of differentiation from the otherwise similar growth of Fränkel's pneumococcus *(q. v.)*.

The streptococcus pyogenes is identical with the *erysipelas coccus*, which was discovered by Fehleisen and Koch in 1881 and obtained in pure culture and successfully inoculated into man and animals by Fehleisen.

The streptococcus frequently gives rise to *mixed infections. It is especially to be feared in primary and in scarlatinal diphtheria,* where its presence often induces fatal sepsis. In many cases of "septic diphtheria," which cause death within a few days, streptococci exclusively are sometimes found; cases of acute choleriform enteritis *with fatal termination* have also been described, in which the dejecta contained large numbers of streptococci in "pure culture." Finally, in many cases of pulmonary

tuberculosis the streptococcus appears to exert an extremely unfavorable influence.

2. In Croupous Pneumonia.

Eberth and Robert Koch found in pneumonic lungs peculiar cocci to which they attributed a causative relation to the pathologic process. Friedländer, conducting examinations upon numerous cadavers, observed a more regular occurrence of cocci.

Friedländer's pneumococci, or **pneumobacilli** [Fig. 33], are small, chiefly oval cells, arranged in twos, threes, or fours, and distinguished by a quite broad *capsule;* water and dilute solutions of caustic potash dissolve this capsule.

[Fig. 33.—Friedländer's Pneumobacillus in Pneumonic Sputum. × 1000. (After *Fränkel* and *Pfeiffer.*)]

Staining. — 1. The cover-glass preparations remain for about twenty-four hours in the following solution:—

Concentrated alcoholic solution of gentian-violet	50.0
Distilled water	100.0
Acetic acid	10.0

Decolor in 1-pro-mille acetic acid and wash in alcohol.

2. The preparation is stained for two to three minutes in anilin-water-methyl-violet solution, then decolored for half a minute in absolute alcohol and washed off in water.

The cocci appear dark blue; the capsules light gray-blue. Frequently several pairs of cocci are seen in one envelope. The diplococcus is *decolored* by Gram's method.

Although Friedländer and others regarded this coccus as the specific exciter of fibrinous pneumonia, this property certainly does not, as a rule, belong to it, for it is neither regularly nor exclusively found in pneumonia, and the inoculation experiments made with it are by no means unassailable, because: (1) croupous inflammation never occurs; (2) the inoculation experiments are too severe procedures. The rare cases of pneumonia in which Friendländer's diplococcus has been found were distinguished by an especially severe course. Furthermore, it has also been found upon the mucous membrane of the mouth, in the saliva and expectorations of other patients, and in the nasal secretions of perfectly healthy individuals, as well as in abscess of the lungs, pericarditis, otitis media, etc.

A microbe very closely resembling this diplococcus is looked upon by Löwenberg, Abel, and others as the cause of ozena *(bacillus mucosus ozænæ)*. [As this micro-organism also occurs in simple atrophic rhinitis without odor, its etiologic significance in ozena is questionable.—Brooks.]

Diplococcus Pneumoniæ (Fränkel-Weichselbaum).[1]

Pneumococcus.—In contrast to that of Friedländer, this coccus is found almost constantly and usually unmixed in fibrinous pneumonia. In the pneumonic lung it occurs most abundantly in the freshest parts of the exudation; furthermore, it is almost constantly present in the diseases complicating pneumonia, such as pleuritis, pericarditis, endocarditis, peritonitis, meningitis, and sepsis.

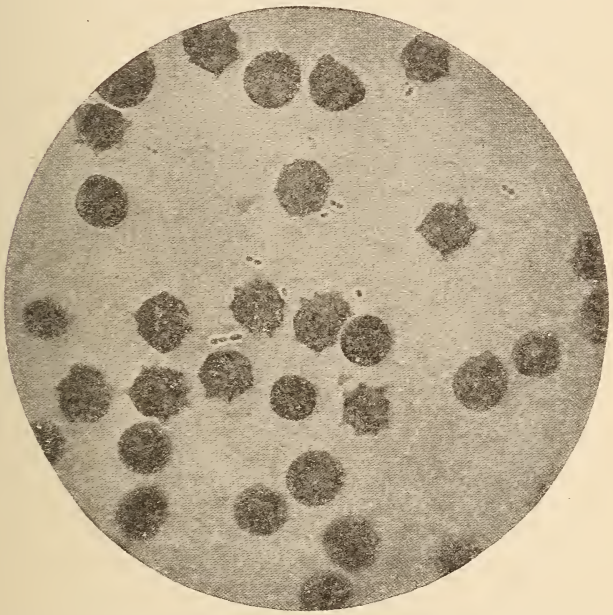
It is almost invariably found in pneumonic *sputum*. In order to demonstrate its presence here it is advisable to wash the expectorated masses several times in sterile water and then to make smear preparations or cultures.

According to the author's experience, the pneumococcus is found in the *living blood* oftener than has hitherto been stated. In a continuous series of 130 cases of pneumonia he found it in the blood 27 times—*i.e.*, in 20.7 per cent. These cases, however, were always of the severest type and generally ended fatally.

The pneumococcus, like Friedländer's coccus, possesses a very distinct mucoid capsule which almost always incloses two ovoid cocci, which are somewhat spindle-shaped at their free ends and united into pairs by their broad bases [Fig. 34]. The author found the diplococcus to be 1.5 to 1.75 μ broad, and 2 to 2.6 μ long. In smear preparations the diplococci are usually isolated, though now and then a chain of two, six, eight, or ten segments is seen. Under such circumstances it is not always

[1] See Plate VII, Fig. 2.

easy to distinguish them from streptococci, because the capsule and the lanceolate form of the cocci are not always sharply defined. In such cases cultivation must be resorted to. Aside from the macroscopic differences, the cultures show important microscopic peculiarities, namely: in preparations made from bouillon cultures the streptococcus is seen in the form of long, twisted chains, while the pneumococci present short, straight threads of eight or ten segments.



[Fig. 34.—Diplococcus Pneumoniæ in the Heart's Blood of a Rabbit. × 1000. (After *Fränkel* and *Pfeiffer*.)]

The *cultures* flourish best in nutrient bouillon, which becomes diffusely clouded by the growth. A slight precipitate collects at the bottom of the culture-tube. Upon agar and blood-serum the colonies appear as delicate, dewdroplike growths.

While the latter are difficult to distinguish from streptococcic colonies grown upon the same media, the growth in bouillon, which usually is *not clouded* by the streptococcus, presents a marked macroscopic difference. Differentiation is easily

accomplished, however, when the cocci in doubt are grown in Petri dishes upon agar mixed with blood. Upon this medium the diplococcus produces a greenish coloring matter, while the streptococcus absorbs the color and as a result the colonies appear to be surrounded by a light halo. In "hanging drop" the pneumococci manifest no motility.

Staining.—1. A cover-glass preparation made from a small portion of the "rusty" sputum is floated for five or six minutes upon an anilin-water solution of gentian-violet, placed for a few seconds in absolute alcohol, and then washed in water to remove excess of stain. The cocci appear blue-black, the capsules colorless and sharply defined against the faintly blue background.

2. If it is desired to make the capsule prominent by a contrast-stain, Wolf's double stain is, according to my experience, the best.

The cover-glass is placed for four or five minutes in fuchsin anilin-water, then for from one to two minutes in a watery, still transparent, solution of methylene blue, and rinsed in water. The dark-blue cocci, surrounded by a rose-colored capsule, contrast very distinctly with the blue-red background.

[The following combination gives a clear image of the capsules of the pneumococcus:—

Dahlia	0.5 gram.
Methyl-green (00 cryst.)	1.5 grams.
Fuchsin (saturated alcoholic solution)	10.0 c. cms.
Distilled water	200.0 c. cms.

Rub up the dahlia and methyl-green in a mortar with part of the water until dissolved, then add the fuchsin, and finally the rest of the water. Prepare the specimen in the usual manner. Flood the preparation (slide) with the stain and heat until steaming. Then lay aside for about five minutes, wash in water, dry, and examine in oil with $^1/_{12}$ oil-immersion lens.

The specimen may be counterstained with 1-per-cent. watery solution of eosin.—BROOKS.]

The staining of the capsule is not as successful in all cases as is stated here. Sometimes it remains unstained even when no error has been made in preparing the specimen.

3. A hasty examination can be made by pouring a few drops of carbol fuchsin upon the cover-glass and warming it for from one to one and one-half minutes over the flame. Wash in water. The cocci are bright red, surrounded by a light capsule.

4. Gram's method *does not decolor* the cocci.

Is Fränkel's diplococcus to be regarded as the specific etiologic factor of croupous pneumonia? It has already been stated

that this coccus is almost constantly present in pneumonia, and occurs most abundantly and almost pure in the most recent foci, from which a conclusion as to its rôle can be drawn. On the other hand, it is also frequently present in the saliva of perfectly healthy persons. It must, therefore, be assumed that under certain unknown conditions an infection of the lungs may occur from this source.

Inoculation experiments with pure cultures have not been sufficiently positive to remove all doubt as to their specific pathogenic nature. Efforts to induce infection by inhalation have always resulted negatively. Subcutaneous injections made into rabbits, mice, and other animals produce fatal septicemia *without* the occurrence of pneumonitic processes. The latter changes, which, to a certain extent, resemble true croupous inflammation, can be induced only by direct injection into the lung itself. The failure experimentally to produce croupous pneumonia in animals cannot, however, be used as an argument against the specificity of Fränkel's cocci, because the susceptibility of the human subject to the pathogenic agents differs from that manifested by experimented animals.

Be this as it may, one fact of great significance remains, namely: Fränkel's diplococcus alone is present in nearly every case of croupous pneumonitis, while Friedländer's bacillus and the streptococcus are but rarely met with.

3. In (Epidemic) Cerebrospinal Meningitis — Diplococcus Intracellularis [Meningitidis] (Weichselbaum).

Weichselbaum, Jäger, and others have found in numerous cases of cerebrospinal meningitis a peculiar coccus which must now be accepted as the almost constant exciter of this disease. Its acceptance as the sole etiologic agent in epidemic cerebrospinal meningitis cannot be entertained for the reason that *sporadic* as well as *endemic* cases of primary meningitis have been observed in which Fränkel's pneumococcus was the only microbe found.

The cocci lie with their flattened surfaces in apposition. A capsule cannot be made out. In size they correspond to the gonococcus *(q. v.)*, though in some instances great variation in size occurs.

Staining.—They are best stained with Löffler's methylene blue solution. Gram's method does not give uniform results.

As the cocci are not infrequently present in small numbers in smear preparations (see "Lumbar Puncture"), *cultivation* is often necessary. They are best cultivated upon *glycerin-agar* in Petri dishes or in culture-tubes upon a slanted medium. After twenty-four to forty eight hours, according to the number of bacteria present in the material used for inoculation, the growth appears as isolated or confluent, transparent colonies resembling drops of water. The individual colonies attain the size of a pin's head, but are only slightly elevated above the surface of the medium. When the inoculated material contains numerous cocci, or the growth is transferred to new media, the surface is covered with a thin, translucent, homogeneous, grayish film.

As the spinal fluid to be examined usually contains but few cocci, it is advisable to use from two to four cubic centimeters for a culture. The superficial colonies, viewed microscopically, are clear and transparent at the periphery; toward the center there is an opaque nucleus.

The cocci grow best at incubator temperature, and not at all at ordinary room temperature. They differ decidedly from the staphylococci in this particular, and also by the fact that they develop poorly upon gelatin. Bouillon is not adapted as a culture medium. The cultures possess but *limited vitality;* they may die in six or eight days. From the gonococcus, which they strongly resemble morphologically, they are distinguished by the fact that the gonococcus cannot be cultivated upon ordinary agar.

Inoculation experiments with animals have thus far given no conclusive results. Multiplication of the microbe does not occur within the animal body. The toxic effects noted are directly traceable to the amount of culture introduced.

4. In Gonorrhea.[1]

The **gonococcus** *(diplococcus gonorrhœæ)*, discovered by Neisser in 1879, is, through the exhaustive researches of Bumm, generally recognized as the specific cause of gonorrhea. It is *constantly* and *exclusively found in gonorrhea* and in identically similar processes, especially *blennorrhea neonatorum*. In the newborn, gonococci were unquestionably found (so far in but two cases) in superficial purulent infiltrations of the dorsum of the *tongue* and of the mucous membrane of the cheeks and of the hard palate (Rosinski-Dohrn and C. Fränkel). The fact that the gonococcus has been proved to be the sole exciter of the following diseases occurring in *connection with gonorrhea* is of the *greatest clinical significance:* Acute serous and suppurative rheumarthritis, pyosalpinx, rectitis, cutaneous abscesses, pleuritis, and ulcerative endocarditis. In gonorrheal rheumatism the author has repeatedly demonstrated the presence of gonococci in the fluid aspirated from the joints.

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[1] See Plate VII, Fig. 3.

The gonococcus was first obtained in pure culture by Bumm, and it has been successfully inoculated upon the mucous membrane of the urethra of two healthy individuals. Consequently, there can be no doubt as to its specific pathogenic action.

Pure cultivation of the gonococcus is now almost universally conducted by the method introduced by Wertheim, in which *blood-serum agar* is employed as a nutrient medium. "Several drops of gonorrheal pus are first mixed with liquid human blood-serum and then two dilutions made from this mixture as directed on page 22. Immediately after inoculation the tubes are placed in a water-bath at 40° C., their contents thoroughly mixed with an equal quantity of liquefied agar (agar, 2 per cent.; pepton, 1 per cent.; common salt, 0.5 per cent.) cooled in the same water-bath to 43° C., and plates poured. The plates are put into a moist chamber and then transferred to the incubator, where they are kept at 37° C. After twenty-four hours a diffuse cloudiness can be seen upon the original plate, and upon Plates I and II isolated colonies are visible to the naked eye which are sufficiently large upon Plate II for transfer inoculation."

Further cultivation in the incubator is successful in tubes containing blood-serum agar (best upon an obliquely solidified surface). A mixture of 1 part of liquid human serum and 2 to 3 parts of meat-juice pepton agar was found to be the most favorable medium. After twelve hours, abundant pure cultures will have developed in the tubes. "Within a few hours there usually begin to develop minute, gray-white points which rapidly enlarge and coalesce to form an extensive, coherent, gray-white, moist, glistening deposit which, by further growth at the margins, produces a delicate, colorless peripheral layer. The 'condensation water' is also covered with a coherent film which, like the remaining cultures, shows numerous gonococci in large sheets."

According to Steinschneider, an increase of the pepton content to 1.5 or 2 per cent. and addition of sterile human urine are conducive to luxuriant growth of the cultures.

Proof that pure growths of Neisser's gonococcus were present in Wertheim's cultures was furnished by several successful inoculations conducted by Wertheim himself (upon paralytics).

In the author's clinic Kiefer's nutrient medium is preferred. It consists of 1 part of ascitic fluid and 1 part of a mixture composed of 3.5 per cent. of agar, 5 per cent. of pepton, 2 per cent. of glycerin, and 0.5 per cent. of sodium chlorid. When pus containing gonococci is spread upon this medium, there will appear after twenty-four hours at 37° C., small, light-yellow to reddish-brown colonies presenting a coarsely granular center, a finely granular peripheral zone, and a serrated margin. If articular or tubal exudate is to be examined, it is advisable if

possible to employ 5 to 10 cubic centimeters for inoculation. Under these circumstances a special nutrient medium is not necessary, since it suffices to mix the blood or purulent fluid with an equal amount of ordinary agar.

A peculiarity of the gonococci—a feature which does not, however, belong to them alone—is that *the majority of them enter the bodies of the pus-cells,* where they multiply in such a manner that they appear wholly to fill up the cell-body and partially or completely obscure the nucleus. The gonococci never penetrate the nucleus itself; they but rarely enter the squamous epithelia, and more rarely invade the cylindric epithelial cells.

The cocci almost always appear in smaller or larger groups, the individuals being *usually united in pairs (resembling a coffee-bean) which lie with their flattened surfaces in apposition.* Now and then are seen four in close contact, which arrangement is produced by fission in two directions of space. The line of division between each pair of cocci is quite broad and always recognizable.

Staining.—A simple and most satisfactory method of staining is that with concentrated watery solution of methylene blue, which is allowed to act upon the preparation for half a minute. Wash in water. Löffler's solution must act for one minute. [A shorter time will do.] Methylene blue is preferable to Bismarck brown, because it stains the cocci much deeper than the nuclei. All of the remaining basic anilin dyes can be employed.

Very beautiful results are obtained with a *freshly diluted* translucent solution of carbol fuchsin [1 part of fuchsin to 8 or 10 parts of water]. If the preparation is allowed to remain in this solution for about two minutes, much more distinct pictures are secured than with methylene blue.

In my courses I have frequently observed that beginners mistook the small prolongations of the polymorphous nuclei of the pus-cells for the cocci. It must, therefore, be strongly emphasized that the latter are, *on an average, only 1 to 1.25 μ in size;* further, that they are chiefly located in the peripheral zone of the pus-cell, accentuating the pale-blue or rose-colored marginal outline of the cell or even supplementing it.

Accurate observation is afforded by *double staining,* in which the cell-body is tinged with a protoplasmic stain and the *cocci* with a nuclear stain.

The cover-glasses are stained for a few minutes in a heated 0.5-per-cent. watery solution of *eosin;* the excess of the dye absorbed with blotting paper; then, *without washing in water*, are stained for one-fourth minute in concentrated alcoholic solution of methylene blue and washed in water. The gonococci are stained intensely blue, and contrast sharply with the eosin-red stained bodies of the pus-cells, the nuclei of which are, as a rule, of a somewhat paler blue than the cocci. *Likewise, the "eosinophile" cells, which are almost constantly present in gonorrhea, are stained with especial beauty* (see "Blood").

A simple and very distinct double staining is secured with *dahlia-methyl-green solution* (1-per-cent. watery solution dahlia-violet, 10 grams; 1-per-cent. watery solution methyl-green, 30 grams), which is allowed to act for one-fourth minute without heating. The cell-bodies are tinged pale red and the cocci bright red; the nuclei reddish green or more of bluish green.

The gonococci are *decolored by Gram's method of staining.*

II. BACILLI.

1. In Tuberculosis.[1]

Ever since Villemin, in 1865, rendered it to a certain degree probable that the products of tubercular disease are transmissible to animals, and Cohnheim, in 1877, successfully inoculated tuberculous material into the anterior chamber of the eye, the conviction that tuberculosis is a genuine infectious disease gained a firmer and firmer hold. This view was confirmed by Baumgarten, who demonstrated the identity of the inoculation nodules of the iris with genuine miliary tubercles. *But the fundamental and indisputable evidence was first furnished by Robert Koch through the discovery of the tubercle bacillus as the sole factor in the production of tuberculosis.* Even though it be admitted that, independently of Koch, Baumgarten observed the regular occurrence of certain bacilli in tubercular foci and inoculation tubercles, to Koch is unquestionably due the credit of having furnished the most absolute and convincing proof of the specific pathogenic nature of the bacillus which bears his name (1882).

Koch proved the constant and exclusive occurrence of the bacillus and successfully conducted its cultivation and inoculation. But what is of greater importance to the physician, he also originated the *"specific" method of staining,* later to be described in detail, which is distinguished by the fact that *the bacilli, when once stained, do not part with the dye upon treatment with nitric acid and alcohol.*

The **tubercle bacilli** are slender, more frequently slightly bent than straight, rods 3 to 5 μ in length (about one-fourth to

[1] See Plate VII, Figs. 4 and 5.

four-fifths as long as the diameter of a red blood-corpuscle); their ends are often slightly rounded. They usually occur singly, seldom in pairs, though occasionally they appear in groups of from five to twelve and more (in the sputum after tuberculin injection; in the urine in urogenital tuberculosis). In stained preparations the rods not infrequently appear to be interrupted by brightly glistening, round or ovoid spaces (Plate VII, Fig. 5), the significance of which is still a matter of conjecture. At present they are quite generally interpreted as signs of degeneration. Fischer and others look upon them as plasmolytic changes. The author is inclined to doubt whether the latter is true in all cases, since it is certain that such features occur more frequently in severe cases with hectic fever than otherwise.

The view that these glistening, round or ovoid spaces occurring within the rods are spores seems to be supported by the fact that a sputum containing bacilli showing numerous clear gaps appears to manifest decidedly pronounced powers of resistance to germicidal influences. In moist media the bacilli are destroyed in 4 hours at 55° C., in 15 minutes at 65° C., in 10 minutes at 70° C., and in 1 minute at 95° C. They resist the action of *dry* heat for hours, and this means is, therefore, not adapted for their destruction. The latter object is best attained by the use of 5-per-cent. carbolic acid or 10-per-cent. lysol solution.

Tubercle bacilli can be grown upon coagulated sheep's-blood serum or glycerin-agar, on which they develop best at 37° C. Their cultivation is attended with some difficulty. A material rich in tubercle bacilli and free as possible from contamination with other bacteria is necessary. This is rubbed into the surface of the coagulated blood-serum. According to Koch, cultures can be obtained from tuberculous sputum in the following manner: After thorough cleansing of the mouth and pharyngeal cavity the patient spits directly into a sterilized Petri dish. If the sputum is found to be rich in bacilli (by stained preparation), the flake is washed in repeatedly renewed distilled water and a portion from the center carefully spread out upon blood-serum or glycerin-agar. The inoculated tubes are now plugged with cotton, and over this is drawn a rubber cap, previously sterilized with sublimate solution, in order to prevent the agar from drying during the fourteen days' exposure to the temperature of the incubator. The colonies are round, smooth, and pure white. Under the influence of sunlight they rapidly die. "According to Koch, if they are placed close to a window they also lose their vitality under the influence of diffused daylight within five to seven days." Since the attempts at cultivation often fail, it is advisable always to inoculate a number of tubes at the same time. (A very favorable nutrient medium is Kresling's neutral meat bouillon, which is as follows: 500

grams of beef-meat to 1 liter of bouillon, with addition of 0.5 per cent. of common salt, 1 per cent. of peptone, and 5 per cent. of glycerin.)

Introduction of the pure culture into animals (field-mice, rabbits, and especially guinea-pigs) by means of subcutaneous injection, inoculation into the anterior chamber of the eye or abdominal cavity, etc., always gives rise to tuberculosis.

Staining.—The alkaline methylene blue solution originally used by Koch is no longer employed.

The following methods of staining are to be recommended:—

1. The **Koch-Ehrlich** method is very reliable, and should be used in all cases where doubt arises.

TECHNIQUE.—The dried cover-glass preparations made from the *purulent* secretion are allowed to float for from twelve to twenty-four hours upon freshly prepared or not too old Ehrlich alcoholic gentian-violet- (or fuchsin) anilin-water solution. They are then placed—without previous washing—for a *few seconds* in a solution of nitric or hydrochloric acid, composed of 1 part of acid to 3 parts of distilled water. In this solution the preparations assume a greenish-blue or greenish-red color, which is the indication for *immediate* washing in 70-per-cent. alcohol and water. If the completely dried preparation is now mounted in balsam and examined under the microscope, the tubercle bacilli only will be seen stained blue or violet (with fuchsin stain, red); *all remaining elements are decolored* by the treatment with the acid and alcohol.

Examination of the preparation is greatly facilitated by the use of a so-called *background*, or *contrast-stain*, which is applied *after* the staining and decoloration of the bacilli is completed. The dried preparation is placed for one or two minutes in a watery solution of Bismarck brown (or methylene blue), then washed in water or alcohol, dried thoroughly, and imbedded in xylol-Canada balsam.

The blue- (or red-) tinged rods now contrast sharply with the brown- (or blue-) stained background.

By heating the above-mentioned staining solution the staining process can be considerably shortened. After from fifteen to twenty minutes the preparation can be taken out and subjected to further treatment with acid, alcohol, and contrast-stain. Too strong heating, however, gives rise to disturbing precipitates of the dyes employed.

2. The staining method of **Ziehl-Neelsen** is also very reliable. An advantage it possesses over the Koch-Ehrlich method is that the principal staining fluid—the carbol fuchsin—is ready for immediate use; furthermore, its staining properties are preserved unaltered for *many months* (see page 34).

The preparations remain in the cold solution for from fifteen to twenty-four hours or *in the heated solution about one-half minute.* Decoloration is secured by washing for a *few seconds* in a 5-per-cent. watery solution of sulphuric acid. After immediate washing in alcohol and water the contrast-staining is made with a watery solution of methylene blue. Dry thoroughly and mount in xylol-Canada balsam.

[The process can be greatly shortened by covering the preparation with the carbol fuchsin solution and then *carefully heating* directly over the free flame until the fluid comes to a boil. *Do not allow the fluid wholly to evaporate during heating,* but renew if necessary.—BROOKS.]

3. Although the Ziehl-Neelsen method can be carried out in a comparatively short space of time and still be depended upon, *Gabbet's* modification of method No. 2 permits of still more rapid staining. The saving of time is secured in the act of decoloration and contrast-staining at the same moment.

Gabbet employed the Ziehl carbol fuchsin solution as the principal stain, allowing the cover-glasses to remain in the heated solution for two minutes. After washing in water the glasses are placed for one minute in solution II, composed of from 1 to 2 grams of methylene blue in 100 cubic centimeters of 25-per-cent. sulphuric acid. Wash quickly and thoroughly in water, dry, and mount in xylol-Canada balsam.

The author has employed this method for many years, not only in his Leipzig courses, but also for years in his polyclinic in Hamburg, and he is convinced that it is extremely convenient and possessed of a high degree of reliability. However, now and then bacilli were found by the Koch-Ehrlich method after they had been missed with Gabbet's stain. A certain amount of caution, therefore, appears to be advisable. Even without heating the carbol fuchsin good staining is secured; but the author prefers heating, because in control tests with two cover-glasses, made at the same time and from the same sputum, decidedly more numerous and deeply stained bacilli were found in the glass stained with the heated solution than in the other.

This is not the place to describe seriatim all the remaining methods which have been proposed for staining the tubercle bacilli. With the above methods success will *always* be attained, provided the specimens which have been prepared contain bacilli. The latter, however, is by no means always the case even in specimens made from unquestionably tuberculous sputum.

It is not rare for an examination of five or six preparations to show but an occasional bacillus; *indeed, in not a few instances* examination of a comparatively large number of specimens may show no bacilli, even though the sputum has a distinctly purulent character and the objective symptoms on the part of the lungs leave scarcely a doubt as to the tuberculous nature of the affection.

In such cases **Biedert's method** sometimes gives positive results.

TECHNIQUE.—Mix a tablespoonful of sputum with two tablespoonfuls of water containing 8 to 10 drops of caustic soda. Boil the mixture under constant stirring, until completely liquefied. Then add about 5 to 10 tablespoonfuls of water, bring to a boil several times, and after eight or ten minutes pour into a conic glass, where it is to remain for two or three days to form a sediment.

In examining for the bacilli, instead of drawing up the sediment with a pipette it is preferable to pour off the supernatant liquid down to the deposit and to make a preparation from the residue after it has been thoroughly triturated. [In this method the sediment often loses much of the adhesive property of the original sputum. It is, therefore, advisable to save some of the untreated sputum with which to fasten the sediment to the slide.—BROOKS.]

The **method of Dahmen** is sometimes of value in demonstrating the presence of tubercle bacilli which escape detection by the ordinary procedures. It is to be looked upon as a modification of Biedert's method, but the addition of caustic soda is omitted and it can be carried out in a much shorter time.

TECHNIQUE.—About half a test-tube of sputum is cooked in boiling water or a steamer for fifteen minutes. By this procedure the proteid substances of the cells coagulate, and, on cooling, fall to the bottom, carrying the bacilli with them. The supernatant fluid, in which there are occasionally a few mucoid coagula extending from the surface downward, is thin and readily movable; this is carefully poured off down to the more or less slight and granular precipitate. The latter is triturated in a dish and can at once be used for cover-glass or slide preparations.

The bacilli contained in this sediment are often arranged in large masses; the individual rods usually appear somewhat less slender, but are otherwise faultlessly stained.

Sedimentation can be greatly hastened by use of the *centrifugal machine* [see Figs. 119, 120, and 121]. This method is especially to be recommended in examining for bacilli in the

urine, where they are often found in large clumps, though not rarely in small numbers.

In many instances the *Van Ketel method* also deserves consideration. Place in a test-tube from 10 to 15 cubic centimeters of sputum, 10 cubic centimeters of water, 6 cubic centimeters of deliquesced carbolic acid, and fill with water to 100 cubic centimeters. Shake vigorously for one minute. After standing for twenty-four hours take up some of the sediment with a pipette and treat in the ordinary way.

If urine is to be examined, 100 cubic centimeters of the latter may be mixed with 6 cubic centimeters of concentrated solution of carbolic acid, then shaken vigorously and allowed to "sediment" in a conic glass [see Fig. 122]. After twenty-four hours the supernatant liquid is carefully poured off and the sediment examined in the usual way for tubercle bacilli (Jolles).

It should not be forgotten that every experienced physician, who is thoroughly familiar with both staining technique and the use of the microscope, will meet with cases of chronic tuberculosis of the lungs in which the most frequent and carefully conducted examinations of the sputum for tubercle bacilli will give negative results. Within the last few years the author himself observed three such cases, and exactly the same experience was reported years ago by von Leyden. It is certain, however, that such cases are of the greatest rarity, and it would unquestionably be wrong for this reason to cast even the slightest doubt upon the reliability of the diagnostic value of bacillary examinations.

In one of the author's cases the autopsy showed but a diffuse—small focused—tuberculosis of the lungs and tuberculous perichondritis of the larynx. The patient expectorated a very moderate amount of chiefly mucoid sputum. In this instance, it is believed, a thorough centrifugation of the sputum would probably have led to a positive result. In a second case the sputum was also chiefly mucoid. Necropsy showed small cavities with intense chronic fibroid inflammation.

The densest masses of bacilli, *real pure cultures of tubercle bacilli,* are met with in such preparations as are obtained from the smooth, yellow, opaque sputum-plugs, *"corpuscula oryzoidea" (q. v.),* or the yellow, cheesy granules of the urine—in vesicorenal tuberculosis. It is advisable for the novice to take a

minute portion of a cheesy mass, derived from cavities in the lungs, for the purpose of staining.

The Pseudotuberculosis or Smegma Bacillus.

Repeatedly *negative* results as to bacillary findings occasionally lead to diagnostic errors, and the same is also true of *positive* results. Contributions citing grave mistakes due to confounding tubercle bacilli with smegma (pseudotubercle) bacilli have been quite numerous in recent years. These bacilli, described by Alvarez and Pavet and Matterstock, when stained by the ordinary methods resemble in every way true tubercle bacilli. They do not give up the dye on short exposure to decoloring reagents, but they usually part with it more readily than do the tubercle bacilli when subjected to prolonged action of alcohol (one hour). As the result of examination of a large number of control preparations we found that the smegma bacilli were generally decolored by careful application of the Koch-Ehrlich method. It would seem, however, that still more caution is necessary, especially since decoloration of carbol fuchsin preparations for ten minutes in 3- to 10-per-cent. hydrochloric-acid-alcohol, as recommended by Honsell, has been attended by erroneous results. According to Pappenheim's researches, *Czaplewsky's method* appears to be reliable:—

Stain with carbol fuchsin and, *without washing,* place for half a minute in concentrated alcoholic solution of yellow fluorescin to which methylene blue in substance has been added in excess, and finally stain with concentrated alcoholic solution of methylene blue. [The smegma bacillus is decolored by this method.—Brooks.]

Runge and Trautenroth recommend removal of fat from the preparation by placing for three hours in absolute alcohol, fifteen minutes in 5-per-cent. chromic acid; then stain with carbol fuchsin and decolor for three minutes in dilute sulphuric acid; finally, for counterstain and further decoloration place for *not less than five minutes* in concentrated alcoholic solution of methylene blue. [The smegma bacillus is not stained by this method.—Brooks.]

As regards *absolute reliability,* we have as yet no method for which such can be claimed. On the contrary, many conscien-

tious authorities look upon animal experimentation only as conclusive.

In sputum examination great care is necessary only in cases of fetid bronchitis and pulmonary gangrene. In urine examinations, on the other hand, caution should be observed *in every case,* particularly since serious surgical procedures are often to be considered in this connection. [When such examinations are in order, a specimen of urine obtained by sterilized catheter, after the external genitalia have been thoroughly cleansed, should always be employed if possible.—Brooks.]

Micrococcus Tetragenus.—In double-stained preparations of tuberculous sputum a greater or lesser number of ordinary cocci or small rods stained with the "background" or contrast-dye are generally seen. As a rule, these are insignificant bacterial admixtures which have either gained entrance to the sputum during its journey through the air-passages or have developed *outside* the body.

Especial importance is to be attached only to the *streptococci (q. v.)* and the coccus above mentioned. This coccus usually occurs singly, rarely in large masses or clumps. It is composed of four rounded segments (about 1 μ in diameter), which are inclosed in a gelatinous capsule. R. Koch, who first found it in the contents of tuberculous cavities, is inclined to attribute to it an *important rôle in cavity-formation.* On the other hand, it is occasionally found in the saliva of perfectly healthy individuals.

It develops upon gelatin plates in the form of glistening, white, slightly elevated spots, and in stab culture [gelatin] along the whole course of the needle track without liquefying the medium. Guinea-pigs and white mice die of sepsis within a few days after subcutaneous injection of cultures.

Staining.—Aside from the anilin dyes, which are used in the ordinary way, the tetragenus is also stained by Gram's method. Double staining can sometimes be secured by the method given for Fränkel's pneumococcus (page 42).

2. In Leprosy.

The bacillus was discovered by Hansen, in 1870, who found it constantly present in leprous nodules. It was subsequently accurately studied by Neisser, and is now generally recognized as the specific causative agent in leprosy.

The leprosy bacilli are slender rods with rounded ends. They are, perhaps, not quite so long as the tubercle bacillus, but, like the latter, are very resistant to the decoloring action of acids and alcohol when once they have taken up the anilin dyes. They also present bright unstained points in their interior, which may possibly be interpreted as endogenous spores (?). These peculiarities entitle this bacillus to an exceptional position.

The bacilli are *constantly* and *exclusively* found in all forms of leprosy, whether the disease be located in the skin, mucous membrane, peripheral nerves, or internal organs, especially the testes and large glandular organs of the abdomen. They are found in the *blood* only in well-advanced cases; on the other hand, Koch and Sticker have emphasized their *frequent* occurrence in the nasal secretion.

Although attempts to cultivate them have *so far been unsuccessful*, there can be but little doubt as to their specific pathogenic nature, for, as has already been said, they are of constant occurrence, and the infectious character of the disease has been proved by successful transference of leprous tissue into animals. *They are, to a certain extent, distinguished from the tubercle bacilli by the fact that they take up the anilin dyes much more readily and part with them more promptly; they are also quite easily and strongly stained with watery solutions, while the tubercle bacilli behave much more stubbornly in these respects* (Baumgarten).

Staining.—All basic anilin staining solutions, even watery solutions, stain the lepra bacilli in a short time. The Koch-Ehrlich and Gram staining methods are to be recommended.

3. In Anthrax.[1]

Anthrax belongs to the diseases most thoroughly investigated from a bacteriologic standpoint. It has not only been determined with absolute certainty that the bacilli discovered in this disease are of constant and exclusive occurrence, and that even the one hundredth generation of the bacilli always produces anthrax, but the germination and growth of endogenous spores have been fully proved upon innumerable occasions. The investigation of these facts is largely due to Koch.

In 1850 Pollender and Brauell found slender rods in the blood of animals affected with anthrax. About ten years later Davaine and Brauell proved the intimate relation existing between the rods and anthrax. They proved that only blood containing the rods was virulent for animals and that blood free of them was harmless. Koch was, however, the first to determine their bacterial nature by means of staining,

[1] See Plate VII, Fig. 5.

cultivation, and inoculation, and he was also the first to observe the development of the endogenous spores into the full-grown bacilli.

According to Koch, the anthrax bacilli belong to the true saprophytes, and only occasionally enter the animal or human body as "facultative parasites." Their development is not dependent upon the human or animal organism. They attack chiefly sheep and cattle, rarely the horse and swine, and never the dog. In the first-named animals infection occurs almost exclusively in grazing places or in stalls when blood and excretions containing spores have been deposited upon the surface. The *enteric form* of the disease is almost always observed in them.

Man is much less liable to infection than animals. The persons usually attacked are cattlemen, tanners, and others coming in contact with the hides, hair, or offal of affected animals. The susceptibility, however, is much less than in the animals mentioned. *As a rule, anthrax makes its appearance in man upon the external skin* (neck, face, and hands) in the form of "*malignant pustule,*" which usually develops rapidly from a small red nodule to an extensive infiltration, upon the surface of which are seen vesicles filled with a serous or sero-sanguineous fluid. In 1872 E. Wagner and Bollinger almost simultaneously described an *intestinal form* of anthrax in human subjects who had died with symptoms resembling typhoid. In the ulcerated infiltrations of the small and large intestine, and in the mesenteric glands as well as in the cerebral hemorrhages, E. Wagner discovered bacilli presenting the same characters as those found in the animal hair with which the deceased had worked.

Cases of **pulmonary anthrax,** described especially by English observers, are of much rarer occurrence. Such cases are seen principally in ragpickers, woolsorters *("woolsorter's disease"),* and workers in paper-factories. The possibility of infection with anthrax by respiration has been experimentally proved by Buchner.

That the disease may also be transmitted to man by the bites of insects is demonstrated by the observations of Huber, who found virulent anthrax bacilli in the bodies of fleas.

The *bacilli* are found in the blood and in the hemorrhagic discharges (from the mouth, nose, bowel, and bladder) of diseased animals, and in the *secretion of malignant pustule* in man (not constant), and also in the blood of the human cadaver, especially in foci of internal hemorrhage. They are hyaline, *non-motile* rods of characteristic form, measuring from about 1 to 1.5 μ thick and from 3 to 5 μ long: *i.e.,* not quite as long as the diameter of a red blood-corpuscle. As a rule, several bacilli are joined end to end. At the point of union of two segments a clear space can be seen. This phenomenon is due to the peculiar

formation of the ends of the bacilli, and in some instances it can be observed even in unstained preparations. It is much more distinctly marked, however, in stained specimens. The extremities of the living bacilli are somewhat enlarged and rounded; in stained preparations they are distinctly cupped upon their circular articular surfaces, so that the comparison with the upper end of the head of the radius is not inapt.

Staining.—They can be stained with all the basic anilin dyes, but too strong dyes or such as readily overstain should be carefully employed. *Watery solutions of Bismarck brown or methylene blue stain cover-glass*

[Fig. 35.—Colony of Anthrax Bacilli, slightly Magnified. (After *Flügge.*)]

[*or slide*] *preparations very distinctly in two minutes.* Occasionally the internal protoplasmic portion and the capsule are plainly marked, but, as a rule, only in bacilli examined directly from the animal body.

In our illustration (Plate VII, Fig. 5) is shown a condition the occurrence of which has been the subject of much controversy, viz.: the frequent inclusion of bacilli within leucocytes.

The bacilli are slightly decolored by *Gram's method.* The cultures grow more luxuriantly on incubation than at room temperature; the optimum is 37° C. Growth ceases below 16° C. and above 45° C.

Upon *plates* those colonies which have reached the surface of the slightly liquefied gelatin are quite large, yellow-white in color, granular in structure, and show at their periphery great masses of twisted and intertwining threads (Medusa head [Fig. 35]). This reticulum is formed

by the luxuriant growth of the anthrax filaments, which develop more rapidly than the advance of gelatin liquefaction and therefore meet with resistance which deviates their growth. This feltlike formation can be very instructively studied in both hanging-drop and impression *(klatsch)* preparations.

The numerous twisted threads can also be seen in [*gelatin*] *stab cultures*. With advancing liquefaction [of the gelatin] the growth gradually sinks as a white, flocculent mass toward the bottom. Upon *agar-agar* the colonies have a peculiar, slightly glistening appearance; on *potato* it grows luxuriantly, forming a whitish, dry coating.

Spore-formation takes place under certain conditions which are as yet insufficiently explained. Free access of oxygen and temperatures not below 24° to 26° C. are necessary requirements for the occurrence of this process. It can be very rapidly and simply secured by growing the bacilli upon potato disks kept at a temperature of 37° C.

The spores appear as rosarylike series of solitary segments which, even in unstained preparations, are characterized by their clear, highly refractive, ovoid form. They possess an extraordinary degree of resistance. While nonsporulating bacilli are rapidly killed by putrefaction, 1-per-cent. carbolic acid, and also by the gastric juice, the spores resist the action of putrefaction for months and 5-per-cent. carbolic acid and the gastric juice for days without alteration in their virulence.

Staining.—The **spores are best stained** by immersing the cover-glass or slide preparation in *hot* carbol fuchsin solution for from twenty to sixty minutes, in order to insure penetration of the dye into even the more resistant spores. The preparation is then placed for one minute in weak hydrochloric acid-alcohol or 5-per-cent. nitric [or sulphuric] acid, which removes the stain from the bodies of the mother-cells. If the preparation is now stained for about one or two minutes in concentrated watery solution of methylene blue or Löffler's solution, the cell-bodies only will appear blue, while the red spores contrast strongly with the blue remains of the mother-cell.

Günther's method is simpler, shorter, and therefore especially adapted for classwork. The specimen is stained in fuchsin-anilin-water solution contained in a watch-glass which is passed back and forth above a flame until the fluid comes to a boil. [This may be further simplified by preparing the specimen upon a slide, then covering the latter with stain, and heating.—Brooks.] The dish is then to be set aside for from one to two minutes. Then, *without washing*, the preparation is immersed for one minute in 3-per-cent. hydrochloric acid-alcohol (preparation side up!). Then follow washing in water and short staining with watery solution of methylene blue. Wash, dry, and mount [in xylol-Canada balsam]. [If slide is used, mounting is not necessary unless the specimen is to be permanently preserved.—Brooks.]

Fiocca recommends the following procedure: About 20 cubic centimeters of a 10-per-cent. solution of ammonia are added to 10 or 20 drops of an alcoholic solution of gentian-violet, fuchsin, or methylene blue.

The mixture is heated to steaming and the preparation placed in it. After from three to five minutes (with anthrax from ten to fifteen minutes) the spores are stained. The preparations are then dipped for a few seconds in 20-per-cent. solution of sulphuric acid, quickly and thoroughly washed in water, and finally stained with a contrast-dye. If the contrast-stain is combined with the acid, as in Gabbet's method, it is advisable to use no more than 10 per cent. of acid. Under these circumstances the preparations should remain in the mixture for from two to three minutes.

Möller advises placing the dry preparations first in chloroform for two minutes, and, after washing in water, for from one-half to two minutes in 5-per-cent. solution of chromic acid; then, after thorough washing in water, for one minute in carbol fuchsin solution which has been brought to a boil once. This is followed by short decoloration in 5-per-cent. sulphuric acid, and, after thorough washing in water, stained with watery solution of methylene blue, which stains the mother-cells. This method gives very good results; it is true that more solutions are used than by the other methods mentioned, but less time is required.

4. In Glanders.[1]

Glanders in the horse is always first localized in the nasal cavities, where it induces catarrh, inflammatory nodules, and ulceration. It gives rise to more or less intense swelling of the lymphatic glands, lymphangitis, cutaneous nodules *(farcy)*, and generally also to *foci in the lungs* [metastases].

The disease is transmitted to man almost exclusively through the agency of the horse. This explains why human glanders is, with rare exceptions (transmission at necropsy!?), observed only in coachmen, hostlers, etc. Slight abrasions offer a route of entry for the virus into the skin, where nodules and furunculous and phlegmonous inflammation develop; or more rarely the infectious material enters the internal organs, of which the lungs and testes are chiefly affected.

The **bacilli** recognized by Löffler and Schütz as the cause of glanders are usually straight and somewhat shorter and thicker than the tubercle bacilli. Their ends are somewhat rounded. They are generally found isolated, rarely in pairs or in large groups. They are frequently surrounded by a delicate halo, which may be interpreted as a capsule. The bodies of the bacilli not infrequently show clear spaces, the significance of which is uncertain. On the other hand, the retention of virulence for months is strongly in favor of the existence of "lasting forms"

[1] See Plate VII, Fig. 1.

(Dauerform). The bacilli are seen free between the cells, but are often found inclosed within the latter. The pathogenicity of the bacilli has been absolutely established by their constant occurrence in all foci and products of the disease, by pure culture, and by the successful transmission of the specific affection by inoculation of the bacilli into horses, field-mice, and guinea-pigs.

Cultivation is successful upon all nutrient media at temperatures from 26° to 40° C. The colonies present a characteristic appearance. Upon blood-serum, which is not liquefied by their growth, they appear as bright, transparent, droplike deposits; upon agar-agar they produce a glistening white layer; upon potato, a yellowish deposit develops which gradually assumes a brownish tinge. No independent motility is observed in hanging-drop cultures.

Staining.—A specific stain, as in the case of the bacilli of tuberculosis and leprosy, has *not as yet been discovered* for the bacillus of glanders. Although the strong basic anilin dyes and especially Löffler's alkaline solution of methylene blue stain the bacilli well, the following special method recommended by Löffler is to be preferred:—

The preparations are stained for five minutes in a freshly prepared staining mixture composed of equal parts of anilin-water-gentian-violet (or fuchsin) solution and 1 to 10,000 solution of caustic potash. They are then treated, *at most, one second* in 1-per-cent. acetic acid to which sufficient watery tropæolin 00 has been added to give it a Rhine wine color. (By this addition, according to Löffler, the dye is wholly removed from the cell-bodies and nearly so from the nuclei.) Finally, wash in water, dry, and imbed in Canada balsam.

The bacilli are *decolored* by Gram's method.

In doubtful cases *inoculation of guinea-pigs* with the suspected secretion appears to be the most certain means of reaching a diagnosis. If nodules, ulceration, and hard tubercles in the testes follow inoculation into the peritoneal cavity, and if the bacilli are also found in these altered foci, the existence of glanders is proved. [As guinea-pigs are not especially susceptible, at least three should be inoculated from active lesions, because in these the bacilli are more numerous and virulent.—Brooks.]

5. In Typhus Abdominalis.

Since the thorough investigations of Gaffky there is no doubt that the bacillus accurately studied by him and previously found by Eberth and Koch in the spleen and mesenteric glands of typhoid cadavers is the causative agent of abdominal typhus. The rods occur constantly and exclusively in abdominal typhus. They have been observed in the blood first by Neuhaus, and in the typhoid stools by E. Fränkel and

Simmonds and especially by Seitz, and occasionally also in the urine. Neumann found them in the urine even from twenty to twenty-one days after convalescence. [Most authorities state that the typhoid bacillus does not usually appear in the urine until quite late. In many cases it persists for a long time (six years!) after convalescence, in which instances it may cause cystitis. The typhoid bacillus has also been found in the bile many years after recovery from the original febrile attack.—BROOKS.] Furthermore, they have been proved to be the sole exciters of many of the suppurative processes observed in the periosteum and serous cavities toward the end of abdominal typhus (Weintraud). Finally, it is worthy of note that they have frequently been demonstrated in the *fresh roseola spots* and in the *living blood* (see below).

The bacilli are quite plump, scarcely a third as large as the diameter of a red blood-corpuscle, and never inclosed within cells. They are characterized by *active independent motility,* which is produced by numerous cilia.

Staining.—They are best stained with carbol fuchsin or Löffler's methylene blue solution. The preparations should remain for five to ten minutes in the staining solution, and then be washed carefully in water. Alcohol should not be used in washing, because the bacilli are easily decolored by it. They are also *decolored* by Gram's method.

If it is desired to *stain the flagella,* it is necessary to observe the greatest care in preparing the specimen, and, *before staining,* to make use of a *mordant.*

A minute quantity of a six-hour-old culture, in which the active motility of the bacilli has been determined in hanging drop, is spread upon a scrupulously cleansed cover-glass and allowed to dry in the air. The preparation is now passed *carefully* three times through the flame, avoiding overheating. The *mordant* described below is now dropped upon it through filter-paper and allowed to remain for from one-half to one minute; this is quickly washed off with water, and the preparation dried, and finally stained for a few minutes (from three to five) with moderately hot gentian-violet-anilin-water solution.

Löffler's mordant is prepared as follows:—

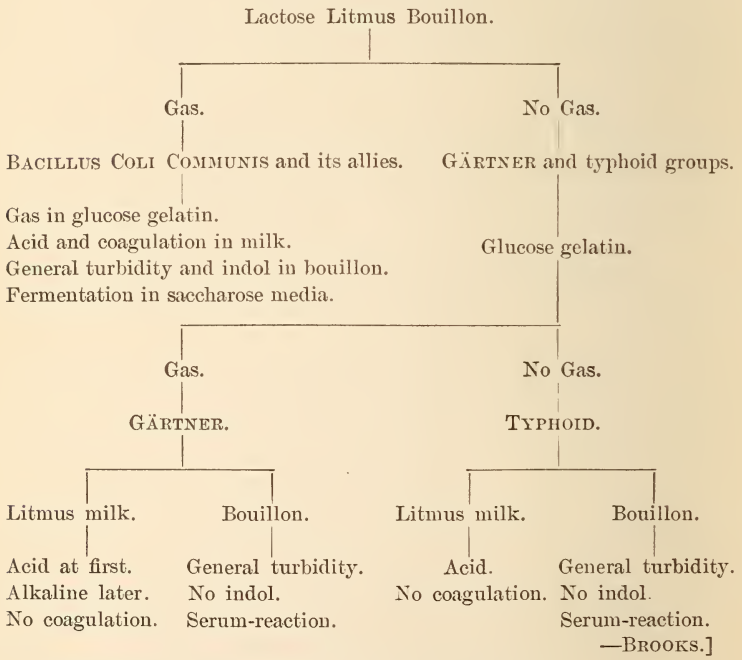
By the aid of heat dissolve 2 grams of tannin in 8 cubic centimeters of water; to this add 5 cubic centimeters of saturated watery solution of iron chlorid and 1 cubic centimeter of saturated alcoholic solution of fuchsin. The mordant should be shaken before use.

As a means of differential diagnosis, the discovery of the bacilli has until recently been of insignificant importance, for the reason that microscopic examination does not suffice, the bacilli can by no means be constantly found in the feces, and especially because the typhoid bacilli are with difficulty distinguished from the colon bacilli.

Both the typhoid and colon bacilli grow more or less luxuriantly upon the above-described nutrient media. Gaffky claimed that the

typhoid bacilli produce a characteristic growth upon potato; the whole surface of the latter is covered at the end of forty-eight hours with a moist and very delicate coating in which the bacilli are present in countless numbers. The colon bacilli, on the other hand, produce a thick, dirty-grayish layer. This difference, however, is not to be relied upon and has been justly questioned by Simmonds and Fränkel. Differentiation of these frequently associated bacteria cannot be made by potato culture alone. The following features only may be considered of importance in distinguishing typhoid cultures from those of the bacterium coli com-

[Analysis of Members of the Coli and Typhoid Groups. (After Eyre.)



—Brooks.]

mune: The typhoid bacilli grow in sterile milk without causing it to curdle even after prolonged action; the colon bacilli, on the other hand, curdle milk within from twenty-four to forty-eight hours. Furthermore, the typhoid bacilli do not produce gas in either bouillon or 2-per-cent. glucose gelatin; the colon bacilli invariably do so. Finally, the cultures of bacterium coli, in contrast to those of the typhoid bacilli, produce a repulsive odor and also give the indol reaction, neither of which phenomena is manifested by cultures of the typhoid bacilli.

Various methods have been recommended for detecting the bacilli

in the stools. The only one of these which appears to be reliable is the culture medium of Drigalski and Conradi.[1]

Prepare a nutrient medium of ordinary gelatin boiled with extract of potato ($^1/_2$ kilogram of potato to 1 liter of water). Before filtration and sterilization add to each 10 cubic centimeters of gelatin 2.5 to 3 cubic centimeters of decinormal solution of sodium hydrate. The decoction should still have a slightly acid reaction. When the medium is desired for use, 1 per cent. of potassium iodid is added to the gelatin and then the necessary plates poured.

While the remaining bacteria develop poorly upon this medium, the typhoid and colon bacilli grow freely. The former appear only after the expiration of forty-eight hours as small, brightly glistening, finely granular colonies resembling minute drops of water, while the colon bacilli are luxuriantly developed at the end of twenty-four hours.

The Gruber-Widal Reaction.

R. Pfeiffer and his pupils demonstrated that the serum of animals immunized against typhoid exerted a specific action upon the typhoid bacilli. Typhoid bacilli which are injected into the abdominal cavity of a guinea-pig are dissolved and disappear if at the same time the serum of an immunized animal is also introduced. It was further shown that Pfeiffer's reaction also occurs in the test-tube. Finally, Gruber and Pfeiffer (Rolle), independently of each other, determined that the sera of human subjects who have passed through an attack of typhoid also exert a specific action upon the typhoid bacilli. If serum from a typhoid convalescent is added to a [bouillon] culture of typhoid bacilli the latter cease their motility, collect in masses (agglutinate), and form a flocculent precipitate at the bottom of the culture-tube. Gruber first recommended this phenomenon for diagnostic purposes. Widal, supported by personal observation, held that agglutination is of diagnostic value not only after recovery from the disease, but also during the attack.

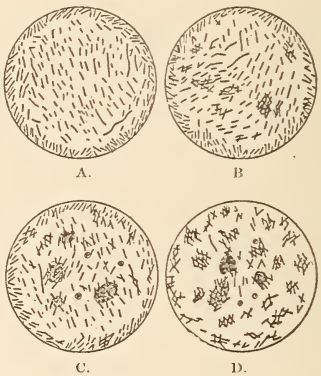
After several years' test of the reaction in a large series of febrile cases, and careful consideration of the numerous publications upon the subject, the author considers it necessary to urge caution in the interpretation of the diagnostic value of the Widal method. There can be no doubt that sera of individuals *not* affected with typhoid and who have never suffered from it may also produce agglutination of the bacilli in bouillon cultures. The sera of typhoid patients, however, give a positive reaction in dilutions which, as a rule, do not suffice with other kinds of serum. According to numerous researches by others, as well as by the author, it may be stated that the Widal reac-

[1] Zeitschrift für Hygiene und Infektionskrankheiten, Bd. xxxix, page 283.

tion can be looked upon as proof of the existence of typhoid only when it is *distinctly positive* in concentration of 1 to 50 [see Fig. 36, *D*].

The reaction may be secured both macroscopically and microscopically.

1. About 5 cubic centimeters of blood are drawn from a distended vein of the arm by means of a sterilized hypodermic needle, and placed in a slanted test-tube until the serum separates. For testing the action of the serum upon typhoid bacilli fresh cultures from six to twelve hours old are necessary. Consequently, the freshest cultures and those in which the sensitiveness toward typhoid serum has been proved to be as great as possible must be constantly at hand for use. [While very young cultures are preferable, those from twenty-four to thirty-six hours old may



[Fig. 36.—Stages in Widal Reaction. (After *Robin*.)]

be used without serious impairment of the value of the test.—Brooks.] The author is accustomed to employ each time several bouillon cultures, using tubes containing 5 cubic centimeters of bouillon. To each of these are added from two to five drops of serum from a pipette; so that the test is made with a concentration of 1 to 50, 1 to 40, etc. The tubes thus treated are kept at 37° C., or at room temperature. When the reaction is positive the bouillon becomes gradually clearer after from three to seven hours, while the bacilli collect in masses and form a layer at the bottom of the tube **(macroscopic reaction).**

2. The **microscopic reaction** is more striking and occurs with greater rapidity. After the motility of the bacilli has been tested in a control preparation 1, 2, and 3 drops of serum (1 drop equals $^1/_{20}$ cubic centimeter) are added successively to 5 cubic centimeters of a fresh bouillon culture or bacterial suspension and then thoroughly mixed by shaking. After each addition of serum a platinum loopful of the culture

fluid is taken for examination. The three preparations correspond to a dilution of 1 to 100, 1 to 50, and 1 to 33 1/3. In this manner it can be noted that the bacilli lose their motility and collect more or less rapidly or even instantly into masses. If clumping does not occur, but the motility of the bacilli continues, the test is *negative*. If the bacilli manifest only a pronounced sluggishness of movement, while clumping is absent, the reaction is not to be depended upon. Under such circumstances the test may be repeated. In this method of examination the degree of concentration can better be controlled.

Only a *positive reaction* obtained after exact attention to the degree of dilution (not below 1 to 50) is of diagnostic value. It should not be forgotten that a positive reaction may also be obtained with the blood of individuals who had typhoid fever years before. On the other hand, a *negative* result cannot be taken as evidence of the nonexistence of typhoid, for in many unquestionable cases of typhoid the reaction may be absent throughout the whole course of the disease even up to the time of death.

[Whenever possible, serum obtained either from coagulated blood, as directed above, or by vesication should be employed. If it be found necessary to forward such material to a distance for examination, capillary glass tubes, into which the serum readily enters, are the most convenient and practical. These are easily made by heating ordinary glass pipette tubing to redness and quickly drawing it lengthwise into a slender thread. The latter can then be broken into convenient lengths. After the capillary tubes are filled with the serum, both ends should be sealed in an alcohol or Bunsen flame. The blood also may be taken in these capillary tubes. The test with this is carried out in the same manner as with serum, but the dilution should be somewhat greater. When either of these methods cannot be carried out, the following procedure may be recommended:—

Carefully disinfect the finger-tip or lobe of the ear and withdraw one or two drops of blood, which are to be received upon a clean glass object-slide. Allow to dry, care being taken that the specimen is not exposed to dust. (The blood collected in this manner can be transported to a distance for examination.) By means of a sterilized platinum-wire loop, dilute and thoroughly mix the blood with *at least 20 parts by volume* of sterilized water. This can be done by dipping the platinum-wire loop in water and transferring to the slide a sufficient number of drops to make approximately a dilution of 1-20 to 1-25. The drops may be placed in a circle around the blood-drop and gradually mixed with the blood, a drop at a time, until a uniform and thoroughly mixed dilution is made. The dilution is now inoculated and *thoroughly mixed* with a minute portion of from twelve to twenty-four hours' growth from an

agar-agar culture of the typhoid bacillus. It is better to have a growth containing the bacilli arranged in long threads, as this form of culture appears to give a more prompt and characteristic reaction. When the mixture of bacilli and dilution is completed (from one-half to one minute usually suffices) cover the fluid with a cover-glass and examine immediately with from a $^1/_6$ to a $^1/_8$ dry objective. If the mixture of bacilli and dilution has been successful, the bacilli will be seen chiefly isolated and in active motion (see Fig. 36, *A*).

A positive reaction is manifested by a more or less gradual cessation of motility and the collection of the bacilli into larger or smaller isolated clumps or connected agglutinations, the bacilli in the masses finally showing but very slight or no motion. The spaces between the masses or clumps may be entirely free of bacilli or nearly so. The time required for the reaction to develop varies from ten minutes to one and one-half hours; as a rule, however, a positive result is obtained in about from fifteen to thirty minutes. If necessary, the observation may be continued for one and one-half hours. If isolated and small clumps are noted at once after placing the specimen under the microscope, these may be due to incomplete mixture, and, hence, should not be regarded as a response to an agglutinating substance in the blood. In such instances a field occupied only by isolated and motile bacilli should be selected and watched for the reaction. Not rarely dilutions of 1-10 to 1-15 may show almost at once an agglutination of the bacilli. Here higher dilutions are in order. In *all cases in which a positive reaction is obtained*—which signifies either that the case is one of typhoid or that this disease has been passed through within a number of months—*the test should be repeated with increasing dilutions, e.g.*, 1 to 30, 1 to 40, 1 to 50, etc., until the reaction is no longer manifested. The degree of reaction can thus be approximated; for example, we may say the blood showed reaction in dilution of 1 to 50, 1 to 100, etc., as the case may be, depending upon the dilution in which the reaction no longer appears. *The higher the dilution in which the phenomenon is manifested, the more positive the result.* As a rule, the better the individual resists a disease the earlier the agglutinating power appears and the more energetic its action (P. Courmont). The Widal reaction is very often absent in cases of typhoid septicemia.

According to Roger, the agglutinating properties may be separated. Heating to 55° C. (131° F.) abolishes the germicidal power, but modifies the agglutinating power very little, if at all. The two substances may also be separated by dialysis. The germicidal substances pass through collodion sacs; the agglutinating substances do not.—Brooks.]

Examination of the Rose Spots.—Different investigators have quite recently obtained positive results in bacteriologic examinations of the fresh roseolæ. Although Neuhaus and Thiemisch had already called attention to the importance of this

adjuvant to diagnosis, we are indebted to Neufeld for its practical application. In fourteen typhoid cases in which the spots were examined by him he obtained positive results in thirteen. After thorough disinfection of the affected cutaneous area, the rose spot is opened and some serum taken with a platinum-wire loop and used for bouillon cultures. The serum should be secured as soon as possible before the escape of blood. Curschmann also speaks highly of this method and claims to have secured fourteen positive results from twenty typhoid patients.

Blood-culture.—Recent researches systematically conducted by my first assistant, Dr. Schottmüller, show that blood-culture is of diagnostic importance.

Twenty cubic centimeters of fresh blood are mixed in five or six tubes of liquefied agar (at 45° C.) and poured into Petri dishes and kept at a temperature of 37° C. If typhoid bacilli are present, colonies will have developed within from forty-eight to seventy-two hours. The colonies upon the surface of the nutrient medium have a grayish color, while those in the depth of the medium appear as blackish points. If on microscopic examination the bacilli in these colonies are found to be motile, they are, without much doubt, typhoid bacilli. Their confusion with colon bacteria is possible only in cases of sepsis connected with disease of the biliary passages, in which condition colon bacilli may enter the blood. For further confirmation the bacilli may be transferred to sugar bouillon. So far, 220 cases have been examined in this manner with *positive* results in 185 = 84 per cent. [The presence of typhoid bacilli in the blood does not necessarily mean a serious infection.—Brooks.]

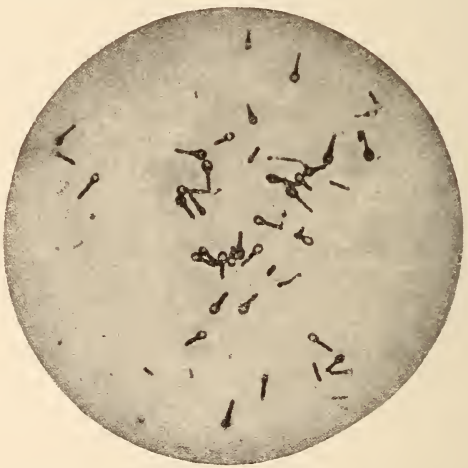
Quite recently my assistant, Dr. Schottmüller, was the first to record observations according to which there can no longer be any doubt that the clinic symptoms of typhoid may be produced by infection with bacilli which are distinguished from the typhoid bacillus by gas-formation and development of alkaline reaction. These cases, in which the Gruber-Widal reaction with the typhoid bacilli is usually absent, but positive with the true infectious agents, are designated by Schottmüller as paratyphoid. Since his observations were published many other authors have reported such cases.

6. In Tetanus.

The bacilli were found by Nicolaier, in 1884, in the sweepings of streets and dwellings and in many varieties of soil. They produce typic tetanus and trismus when inoculated into animals, particularly guinea-pigs. They were also discovered by Rosenbach in cases of human tetanus and by Pfeiffer at the focus of infection in tetanus neonatorum. Kitasato obtained pure cultures of the bacilli, which are *obligate anaërobic*, by

inoculating tetanus pus upon solidified blood-serum and then exposing the culture for one hour at 80° C. in a water-bath in order to destroy all vegetative bacteria. From these cultures, which now contained nothing but *spores*, gelatin tubes were inoculated and poured into dishes into which hydrogen-gas had been introduced. Pure cultures of tetanus bacilli develop at from 18° to 20° C.; incubation temperature favors their growth, which ceases below 14° C.

Since the spores of other bacteria may be present in the original material, it is advisable to make *animal tests* in addition to plate cultures. If some of the suspicious wound secretion is introduced beneath the skin of guinea-pigs or mice, tetanic phe-



[Fig. 37.—Bacillus Tetani. × 1000. (After *Fränkel* and *Pfeiffer*.)]

nomena will appear within the first twenty-four hours, provided traumatic tetanus is actually present.

The bacilli are delicate rods, sometimes forming threads, sometimes occurring in clumps, but usually single. Only the nonsporulated forms manifest slight independent motility. The spores are round and generally polar [Fig. 37].

Inoculation of pure cultures into animals is most successful in mice, guinea-pigs, and horses. A specific toxin is formed which causes the characteristic phenomena of tetanus, while the bacilli themselves disappear entirely. Pure cultures of tetanus freed of the bacilli act in a similar manner. For this reason the tetanus bacilli are classed with the "toxic" bacteria. The blood-serum of artificially immunized animals neutralizes the action of tetanus toxin.

Staining.—The *bacilli* stain with all basic anilin dyes; Löffler's methylene blue solution is especially to be recommended. For staining the *spores* it is necessary to employ the method given for those of anthrax (see page 58).

7. In Cholera Asiatica.

The **comma bacillus,** discovered by Koch in 1883 and found by him to be present exclusively in cases of genuine cholera, is now universally recognized as the specific cause of Asiatic cholera.

It is found in the intestine and feces, but *never* in the blood or the organs of the body. A feature of diagnostic importance is that it often occurs in enormous numbers in almost pure culture in the well-known "rice-water" or "souplike" stools. The more feculent the stools, the less numerous the bacilli. The bacilli are slightly bent, commalike rods, about half as large as, but distinctly thicker than, tubercle bacilli. Sometimes they are very decidedly bent, almost horseshoe-shaped, or they resemble a large Roman letter S, which is formed by their peculiar end-to-end union with each other. Otherwise they usually occur singly, rarely in long, undulating threads. The latter are, as a rule, involution forms, as may be inferred from the spirillum-like forms observed in cultures grown under unfavorable conditions. The latter phenomenon has led to their classification with the spirilla.

The cholera bacilli are further distinguished by their very *active motility.* The occurrence in them of endogenous spore-formation can certainly be denied. The arthrogenous sporulation described by Hüppe is improbable, because of the readily perishable nature of the bacilli. *Drying* often destroys their virulence within a few hours, and with certainty after from one to two days. Virulence is preserved for months in a moist state. *Of practical interest is the fact that normal (acid) gastric juice quickly kills them,* and that they are also destroyed by putrefaction and disinfecting solutions (even $^1/_2$-per-cent. carbolic acid).

Infection apparently occurs through the agency of food, which either contains the bacilli (drinking-water, milk, fruit, etc.) or is contaminated by bacilli from soiled hands, etc., usually while eating.

As cholera Asiatica never occurs in animals, successful inoculation experiments would appear to be hopeless. Nevertheless, Koch succeeded in producing in guinea-pigs a severe intestinal affection by neutralizing the acid of the stomach and arresting peristalsis by opium. In man cholera has repeatedly been induced by accidental or intentional (von Pettenkofer and Emmerich) transference of cultures of the bacilli. Fortunately, however, these cases of "laboratory cholera" were, as a rule, *not* fatal. *That infection occurring in this manner may end fatally after pursuing a course presenting the typic picture of genuine cholera* is strikingly shown by the tragic case of Dr. Oergel, of Hamburg, reported by Reincke. In all these cases virulent comma bacilli were found in the colorless dejecta of the patients.

Animal experiments can hardly be considered as of diagnostic value. For its performance take a platinum-wire loopful of the surface growth of an agar culture, mix in sterile bouillon, and inject into the abdomen of a guinea-pig of about three hundred grams' weight. The animal, which is very susceptible, usually dies in from twelve to fifteen hours with great fall in temperature. According to R. Pfeiffer, the toxin is intimately united with the cell-bodies of the bacilli.

Staining.—The bacilli stain with all basic anilin dyes, best with fresh dilute carbol fuchsin solution, made by adding a few drops of Ziehl's solution to water. The preparation should be stained with this for five (at most ten) minutes. [The bacilli can be very rapidly and distinctly stained with a *hot, dilute* solution of carbol fuchsin, made by adding 1 part of Ziehl's solution to 9 parts of water.—Brooks.]

The bacilli are decolored by Gram's method.

In the absence of a specific stain, a positive diagnostic decision cannot be made from the microscopic appearance of the bacilli in preparations made from the stools. The resemblance of the bacilli to many rods and spirilla found in the feces can readily lead to misinterpretation, especially by the *inexperienced* examiner.

According to R. Koch, however, from the material examined in the Institute for Infectious Diseases, a diagnosis could be made from the microscopic appearances in about three-fourths of the cases of genuine cholera. Of course, the diagnosis was always confirmed by cultures.

For **pure culture** of the bacilli it should be remembered that the nutrient medium must have a distinctly alkaline reaction. According to Dahmen, this is secured by adding 1 gram of soda to 100 cubic centimeters of accurately neutralized boiled gelatin.

For cultures upon *gelatin plates* several of the characteristic mucous flocculi (which give to the stools the rice-waterlike appearance) are carefully shaken in gelatin previously liquefied and cooled to 37° C. From this the usual dilutions are made and poured into several Petri dishes and kept at high room temperature (from 20° to 24° C.). After from twenty to twenty-four hours distinctly granular colonies can be

seen upon the plates. With about 100 magnification these appear as though composed of small, strongly refractive glass fragments. Liquefaction of the gelatin surrounding the colonies produces a small, funnel-shaped depression, at the bottom of which the mass of bacteria collects. The colonies glisten brightly when the objective is purposely forced *below* the proper focus, and become dark when focused too high. Of the numerous bacteria occurring in the feces under normal and pathologic conditions, only a few forms grow in nutrient gelatin. They are distinguished from the comma bacilli by the fact that they usually *do not liquefy the gelatin.*

Growth in *gelatin stab culture* is characterized by the appearance of a funnel-shaped widening of the upper portion of the stab, and a delicate spiral twisting of the lower, narrow end of the needle-track. Upon *agar* the colonies appear in the form of a moist, glistening, grayish-white layer. *Bouillon* is strongly clouded by their growth.

To hasten the diagnosis of cholera (and also for use in material that, in addition to numerous others, contains comparatively few cholera bacilli) R. Koch has offered the following method:—

Pepton Culture.—A small portion of the suspected stool is placed in a test-tubeful of pepton solution (pepton, 1 per cent.; sodium chlorid, 0.5 per cent.), and then incubated at 37° C. Because of their great necessity for oxygen, the rapidly developing cholera bacilli very quickly collect upon the surface. If a drop from the *surface* of the pepton solution is taken about from six to twelve hours after inoculation: *i.e.*, when the liquid just begins to cloud and before the pellicle has formed, there will be found (in cholera) either a pure culture or a mixture containing a very large number of cholera bacilli (Dunham-Dunbar-Schottelius method). In every case pure cultivation should be further conducted upon gelatin or other nutrient media.

Although the appearance of the colonies upon *agar plates* is less characteristic than in gelatin, demonstration is far more rapid by this method because the plates are kept at 37° C. If a drop from the surface of a *pepton culture* is spread with a platinum-wire loop upon an agar plate[1] development of the colonies takes place within from eight to ten hours.

Finally, the **cholera reaction** is of some diagnostic significance. In albuminous nutrient media cholera bacilli develop (as do many others) indol and nitrites. If pure hydrochloric or sulphuric acid is added to such a culture, nitric acid is liberated and a distinct rose or Burgundy-red color develops. The reaction can be secured with a *pepton culture* at the end of twenty-four hours. Salkowski demonstrated that this phenomenon is dependent upon the well-known **nitroso-indol reaction.**

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[1] It is advisable first to free the agar of the so-called "condensation water" by evaporation. This can be done by allowing the liquefied agar to solidify in Petri dishes and then placing the latter in the incubator for some time.

An unquestionable conclusion is possible only by consideration of the characteristic features of the cultures. When it is necessary to identify as cholera a vibrio cultivated from the feces, Pfeiffer's reaction is the most certain aid. This corresponds in every way with the test described in connection with the diagnosis of typhoid (page 63).

8. In Diphtheria.[1]

The bacilli discovered in diphtheritic membranes by Löffler and secured by him in pure culture have been *unquestionably* proved to be the specific exciters of diphtheria. Their diagnostic significance has been repeatedly confirmed by other investigators. The bacilli occur principally in and upon the membranes: in fresh cases often in pure culture, in older cases mixed with other bacteria. Not infrequently they are found in the pharyngeal mucus of patients. The "nests of bacilli" sometimes seen in stained preparations made from the membrane often suffice for diagnosis; in other instances, where many other bacteria are also present, diagnosis must be established by cultures.

The diphtheria bacilli occur in a small and a large form. The "**pseudodiphtheria bacilli**" may be mistaken for them, since they are arranged in a similar manner and also show a certain degree of segmentation. They are usually *smaller* and *thicker* than the true diphtheria bacilli, and do not present the clublike swellings at their ends. When cultivated in alkaline bouillon true diphtheria bacilli produce a distinct acidity, which is not present in the cultures of pseudodiphtheria bacilli. In doubtful cases positive animal experiments (see below) are of importance.

For the **demonstration of the bacilli** it is best to scrape some of the membrane off with a sterilized platinum-wire loop and make a dry preparation, or a piece of the loosened membrane may be quickly wiped over the surface of a cover-glass [or slide]. For the *preparation of cultures* a platinum-wire loop is drawn once or twice over the slanted surface of coagulated blood-serum[2] or agar which is sprinkled with fresh human blood (taken prefer-

[1] See Plate VIII, Fig. 8.

[2] Löffler's blood-serum: Three parts of sheep's and beef's serum and 1 part of beef bouillon containing 1 per cent. of glucose; sodium chlorid, 0.5 per cent., and 1 per cent. of pepton.

ably from a vein of the arm with a Pravaz syringe). After twelve (often after eight) hours in the incubator the characteristic colonies appear as stearin-white, fine and coarse drops. At first they are isolated, but subsequently coalesce.

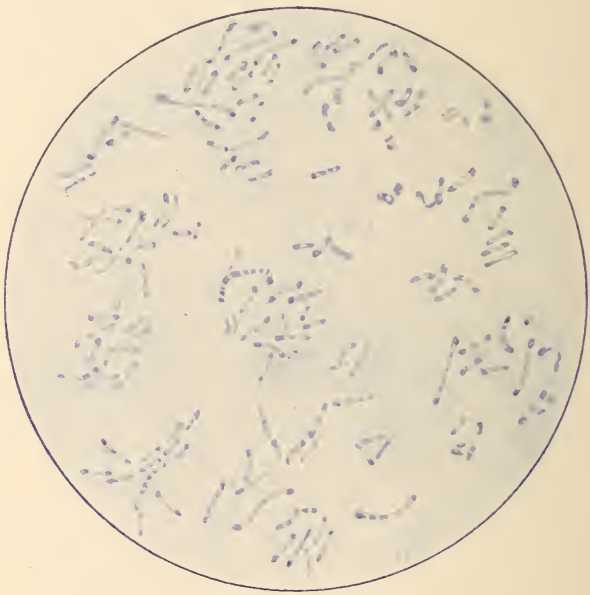
[The *diphtheria bacillus* can sometimes be found in preparations made from portions of the diphtheritic membrane. The specimen is made in the same manner as in sputum examinations: *i.e.,* spread into a thin layer upon a slide and dried. The dried preparation can be stained with cold Löffler methylene blue solution, for from two to five minutes, then washed in water, dried, and examined in cedar-oil with $^1/_{12}$ oil-immersion lens. When possible, cultures upon an appropriate nutrient medium (blood-serum) should be made from the throat, and the resulting growth examined after from twelve to eighteen hours. The following directions for making a diphtheria culture from the throat are abridged from a circular issued by the Bacteriological Department of the New York City Board of Health:—

The patient should be so placed as to offer a good view of the throat. Depress the tongue and with a sterile cotton swab rub gently, *but freely,* against any visible exudate, *revolving the swab between the fingers* so as to bring all portions of the swab in contact with the mucous membrane or exudate. Withdraw the cotton plug from the culture-tube, holding it so that the portion withdrawn from the tube does not come in contact with the fingers or any other substance. Carefully insert the swab in the culture-tube and *rub it gently, but thoroughly,* back and forth over the entire surface of the culture medium for at least half a minute, revolving the swab so as to bring all portions of it in contact with the surface of the culture medium. *Do not allow the swab to touch anything except the throat of the patient and the surface of the culture medium.* Do not push the swab into or break the surface of the culture medium in any way. Do not use tubes in which the culture medium is contaminated, liquefied, or dried up. Replace the plug in the inoculated tube, mark for identification, and place in the incubator (99° F.). Unsatisfactory cultures, showing insufficient growth or contamination, usually result from failure to follow carefully these directions.

In examining the cultivated bacilli, a minute portion of the growth is transferred to a clean slide by means of a platinum

wire, thoroughly mixed with a small drop of water, then spread thinly over the surface of the glass, and dried by aid of gentle heat. Stain for two or three minutes with cold Löffler methylene blue solution, or with heated dilute carbol fuchsin (Ziehl) solution (1 part to 9 of water), wash in water, dry, and examine in cedar-oil with $^1/_{12}$ oil-immersion lens.—Brooks.]

The **bacilli are stained** for one or two minutes in heated Löffler's alkaline methylene blue solution, or one or two minutes



[Fig. 37*a*.—Bacillus Diphtheriæ. × 1000. (From drawing by E. L. Oatman, M.D.)]

in dahlia methyl-green, or three minutes in fresh, concentrated alcoholic gentian-violet-anilin-water solution. The latter stain is to be preferred, because Gram's method can afterward be used if required. In this connection, according to Plaut, washing with alcohol should not be continued until complete decoloration. Anilin-oil is better than alcohol. [The bacilli can be beautifully and rapidly stained with dilute heated carbol fuchsin (Ziehl) solution (1 part of Ziehl to 9 parts of water). Wash in water, dry thoroughly, and examine.—Brooks.]

The *nonmotile rods* are usually about as long, but twice as broad, as tubercle bacilli. The ends often appear swollen in clublike form. In stained preparations, especially those colored with Löffler's methylene blue solution, the bacilli generally present very distinct segmentation. This frequently gives to them a somewhat granular appearance, which, in addition to the so-called "clubbed form," is, to a certain extent, characteristic of the bacilli.

Virulent bacilli have occasionally been found in the mouths of healthy and slightly indisposed individuals. What is of more importance from a practical point of view, however, is the fact that they have been observed in the oral cavities of convalescents for days and even weeks after disappearance of the membranes (Escherich). According to Flügge, when deposited upon toys, utensils, and articles of clothing, they retain their virulence for from four to six weeks. If protected from complete desiccation, strong light, and the action of putrefactive bacteria, they may live for from six to eight months. Damp soiled clothing kept in a poorly lighted, cool cellar is said especially to favor preservation of their vitality. Infection occurs chiefly from mouth to mouth, by expectoration, and through the agency of contaminated objects. In the form of *dust* the bacilli are not infectious. Whether they are capable of development upon meat, milk, and broths and thus produce infection is still a mooted question.

The observations concerning the occurrence of Löffler bacilli in xerosis and many forms of conjunctivitis which clinically are not to be considered conjunctival diphtheria require more thorough explanation and investigation.

Transference of the bacilli to animals (one to two loopfuls of a fresh culture subcutaneously), especially to the very susceptible guinea-pig, does not produce diphtheria, but an unusually severe intoxication of which the animals die in from one to four days. If the period of illness is more protracted, paralysis in every way resembling postdiphtheritic paralysis is observed. Löffler's assumption that the multiplication of the bacteria at the point of infection is accompanied by the development of a toxin which is profoundly injurious to the body has been universally confirmed. A further danger from the activity of the bacilli is the epithelial necrosis produced by them, which opens the way for the entrance of other bacteria, especially streptococci.

Additional proof of the specific significance of the diphtheria bacilli is furnished by the artificial immunization tests of Behring, which confer upon otherwise susceptible animals a pronounced degree of "toxin immunity" to the severest attempts at infection.

9. In Influenza.[1]

R. Pfeiffer has described delicate bacilli as the causative agents of la grippe or influenza. The assumption as to their specific pathogenicity has been confirmed by their morphologic peculiarities, their exclusive occurrence in influenza, and by pure cultivation. Successful inoculation experiments, however, have thus far not been obtained; but this is not surprising when it is remembered that no single species of animal is spontaneously attacked by la grippe. On the other hand, many animals—*e.g.*, rabbits—are susceptible to the toxic effects; they die with dyspnea and paralysislike prostration. In their *cultivation* Pfeiffer at first met with great difficulties, which were overcome only when he employed sterile blood sprinkled upon the surface of slanted agar and then rubbed into this medium the grippal expectoration. This was followed by luxuriant growth of colonies, which could be further cultivated at will. Hemoglobin is indispensable for the growth of the colonies. [According to Rymowitch, the **Koch-Weeks bacillus**, found in the mucus of acute conjunctivitis, and the influenza bacillus are identical.—Brooks.]

For the **preparation of pure cultures** Pfeiffer recommends the following method: A fragment of sputum is rubbed up thoroughly in from one to two cubic centimeters of bouillon in order to distribute the bacilli as much as possible, and to secure the formation of separated colonies. The culture is then made as above described.

All the colonies show a decidedly glasslike transparency. They are *aërobes*, grow best at 27° to 42° C., and are developed after twenty-four hours. In bouillon or upon blood-agar they retain their virulence for from fourteen to twenty-eight days; they also retain their vitality in moist sputum for the same length of time (Pfeiffer), but they are very susceptible to the action of drying.

The bacilli often appear in the **sputum** in pure culture; they have also been demonstrated by Pfeiffer in parenchymatous tissues in influenza pneumonia, and by others in influenza encephalitis and meningitis; finally, also in the blood.

In order to demonstrate microscopically the bacilli in the sputum the latter must be examined fresh. The sputum should be spread out in a sterile glass dish and a portion taken from the middle of the **purely purulent** part. In fresh, uncompli-

[1] The illustration (Plate VIII, Fig. 9) was made from an original preparation furnished the author by R. Pfeiffer.

cated cases the bacilli will often be found, as Pfeiffer first demonstrated (and the author could confirm), in great numbers and often in pure culture. The bacilli usually lie in *clumps* in the mucoid basement substance of the sputum, partially also within the bodies of the pus-corpuscles. They are generally from two to three times as long as broad. The ends are rounded; sometimes two short bacilli lie so close together that they greatly resemble diplococci. They are *nonmotile* and have no capsule.

The bacilli are usually very numerous at the beginning of the disease. With progress and subsidence of the malady the number of *free* bacilli becomes less, while the pus-corpuscles appear to be stuffed full of them. Degenerative forms frequently occur at this stage; the bacilli appear fragmented, stain badly, etc.

Staining of preparations is best done with freshly prepared, dilute carbol fuchsin solution, which should be allowed to act from ten to twenty minutes. Wash in water, dry, and imbed in Canada balsam. [If slide is used Canada-balsam imbedding is not necessary unless it is desirable to preserve the specimen permanently. On the contrary, the immersion oil can be placed directly upon the smear and the specimen examined. Staining can be greatly hastened by heating the fuchsin solution.—Brooks.] The delicate rods, free and intracellular, show a partly uniform staining and partly a polar staining, which not infrequently lead to their being mistaken for diplococci. They are *decolored by Gram's method.*

10. In Oriental Bubonic Plague.

The bacillus of bubonic plague was discovered by Yersin, and its regular occurrence in the swollen lymph-glands and the pus from these, as well as in the lungs, liver, spleen, and blood, has been positively proved. The germs enter chiefly through the skin and lungs, although infection may also occur through the intestine.

Mice, rabbits, guinea-pigs, and especially rats are susceptible; indeed, the latter are said to play an important rôle in the spread of the plague. In the last epidemic in Canton the outbreak in human subjects was preceded from two to three weeks by great mortality among rats, and this phenomenon was repeated in each newly attacked portion of the city.

The bacilli are short, thick, scarcely motile rods, with rounded ends, which stain intensely on treatment with basic ani-

lin dyes (polar staining). They do *not stain with Gram's* method.

Cultivation is readily secured upon ordinary nutrient media at room and body temperature. In gelatin they grow as small, round, finely granular colonies. Upon agar they form a grayish-white iridescent film. *Bouillon* is *not clouded* by their growth; flocculi of bacteria collect at the bottom of the tube, in this respect offering a certain similarity to streptococci.

Yersin found that their growth occurred best in 2-per-cent. pepton solution containing 1 to 2 per cent. of gelatin. Gas and indol development are absent.

Spores have *not* been observed. Hence their slight degree of resistance. Heating at 80° C. kills the bacilli in from ten to twenty minutes, and at 100° C. in a few minutes; treatment with 1-per-cent. carbolic acid kills them in one hour.

According to Haffkine, injection of dead cultures of bubonic plague bacilli supplies a certain degree of protection. Infection with the [live] bacilli in nonfatal doses confers immunizing properties upon the blood-serum.

Bacterium Coli Commune and Bacterium Lactis Aërogenes.

In connection with the pathogenic bacilli we will briefly discuss:—

1. The **bacterium coli commune** (Escherich). This bacillus has in recent years attracted much attention as the causative agent in purulent (perforating) peritonitis, angiocholitis, and many forms of cystitis. The author has repeatedly found this bacterium in pure culture in cystitic urine; also in the inflammatory exudate of the gall-bladder and gall-ducts (at operation and at necropsy); finally, a number of times also in the blood of patients suffering from severe—fatal—cholangitis, and in a few rare cases of puerperal fever in which the bacilli, combined with streptococci, appeared in the blood and metastatic foci. Schmidt and Aschoff found the bacterium coli commune nine times in pure culture in fourteen cases of pyelonephritis. It is constantly found in the colon of children and adults as well as in all intestinal discharges.

The bacilli appear as delicate or plump, short rods of an average thickness of 0.4 μ. They possess slight independent

motility, which is effected by means of one or more polar flagella. The bacilli often occur in pairs. They *stain* readily with the ordinary methods; they are *decolored* by Gram's method.

In *pure culture* upon gelatin plates the bacilli grow in a form resembling typhoid bacilli, producing small, whitish spots in the depth, and upon the surface colonies with serrated margins. They *do not liquefy* the medium. They render bouillon cloudy, and grow upon agar in the form of a gray-white film and upon potato as a yellowish, moist deposit (see page 26). Points of differential diagnostic importance are: they ferment glucose solutions with profuse production of gas (CO_2), they quickly coagulate milk (at incubation temperature) with intense formation of acid, and, finally, when cultivated in pepton nutrient media they produce the *nitrobenzol reaction*, which can be made to appear as a distinct rose coloration by addition of 1 cubic centimeter of 0.02-per-cent. solution of potassium nitrite and a few drops of chemically pure sulphuric acid to 10 cubic centimeters of the bouillon culture (see also page 71).

2. The **bacterium lactis aërogenes**, also first described by Escherich, resembles in many ways the bacterium coli commune. It occurs regularly in the feces of infants and not infrequently in the stools of adults. It occasionally manifests pathogenic properties. Its recent demonstration by Heyse as the exciter of *pneumaturia* is a most interesting fact. In this instance the infection of the bladder was probably induced by catheter; the bacilli were found in the *foamy* vaginal secretion, in the feces, and also (postmortem) in the contents of the colon.

It appears in the form of quite thick, short rods, which are not infrequently united in pairs, thus presenting the appearance of a diplococcus. The rods are *nonmotile,* do *not* form spores, and are *decolored* by Gram's method, in which particulars they resemble the bacterium coli.

In pure culture perceptible *differences* between this bacillus and the bacterium coli are observed.

In plate cultures the bacterium lactis produces a very dense, glistening, white layer. In stab cultures a chain of pearllike white colonies appears, and when the inoculation opening is at once closed they also form, after twenty-four hours, numerous gas-bubbles, the size of a lentil, which rapidly increase in size and widely distend the gelatin. The cultures are odorless. Upon *potato* appear grayish-white, moist, glistening deposits several millimeters thick, containing numerous gas-bubbles, which are not present in such cultures of bacterium coli. Fresh *agar* is entirely covered, after four hours, with a thick, white layer, such as is

observed with the bacterium coli only after a much longer time. After twenty-four hours *milk* is curdled in large lumps, which separate from the clear whey. Gas-formation is much more energetic here than is the case with bacterium coli.

In Dysentery.

Various authors—especially Shiga, Flexner, and Kruse—have recently demonstrated the constant presence of certain bacteria in the dejecta and in part also in the organs of large numbers (epidemics) of dysenteric cases. The researches of these investigators are of much greater significance than those of former years, because the cultivated bacterium is agglutinated by the blood of dysenteric patients in the same manner as the typhoid bacillus in the Widal reaction. In this manner the etiologic rôle of the *"bacillus dysenteriæ"* in at least a number of epidemics is assured.

This bacillus is described by Shiga as follows: Those bacilli found in dysentery by the other authors mentioned differ only in unimportant details:—

It is a bacterium closely related to the typhoid bacillus and bacterium coli. It is morphologically identical with these. It is decolored by Gram's method of staining. It is moderately motile. It forms pale-yellow, finely granular colonies in gelatin, and does not liquefy this medium. On agar it produces a bluish, transparent film. There is no formation of gas in sugar-agar. Upon potato it shows after twenty-four hours a scarcely visible, whitish layer; later it produces a brownish, mosslike pellicle. Bouillon is diffusely clouded; no indol reaction. There is acid-formation in litmus-milk, but milk is not coagulated.

[Quite recently Duval and Bassett have isolated this bacillus from the dejecta of forty cases of *summer diarrhea in infants* and also from scrapings of the intestinal mucosa at necropsy, and in one case from the mesenteric glands and liver. In this affection the bacillus was first isolated by Duval, Bassett subsequently aiding him in his researches. The investigations of Duval have been confirmed by Spronck, of Holland. The organism is agglutinated by the serum of patients suffering with the disease.—Brooks.]

III. SPIRILLA.

Spirochæta Obermeieri in Febris Recurrens.

In *all* cases of recurrent fever, and in this disease *only,* there is *regularly* found in the blood a spirally twisted, uniformly delicate bacterium which was described in 1873 by Obermeier and spoken of with all positiveness as the causative agent of the disease. The spirilla can readily be seen in the fresh, unstained blood with a linear magnification of from 350 to 450. Their presence is usually manifested by *active spiral progressive move-*

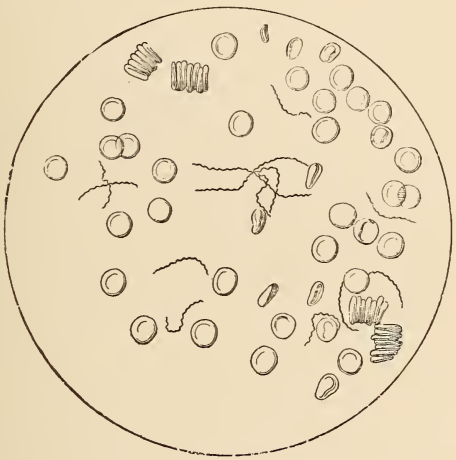


Fig. 38.—Spirochæta Obermeieri. × 380.

ments, which are produced with great violence and often thrust aside whatever blood-corpuscles may be in the way. Although they are generally observed singly in the field of the microscope, it is not rare to see them united in larger or smaller groups. The microbes vary greatly in length; they measure from two to five times the diameter of a red blood-corpuscle. Their ends are usually somewhat more slender than the rest of the thread. *They are always found in the blood,* but never in the secretions or excretions. They appear shortly before or at the beginning of the febrile attack, increase greatly during the latter, and disap-

pear entirely with the subsidence of the fever, to reappear and repeat a similar cycle with each new relapse.

They have not as yet been cultivated, although inoculations with the blood containing the spirilla have been successfully practiced upon man and monkeys. The mode of infection is still doubtful, but the disease is perhaps transmitted by fleas.

Demonstration of the spirilla in the blood renders the diagnosis *absolutely positive.*

Staining is entirely unnecessary; if, however, it is desired, the dried blood-specimens may, according to Günther, be moistened for ten seconds with 5-per-cent. acetic acid (to remove the hemoglobin from the red blood-cells), and then stained for ten minutes with gentian-violet-anilin-water solution. They also readily and quickly take up all other anilin dyes in watery solution.

2. STREPTOTHRIXIÆ.

These occupy a position midway between bacteria and thread fungi. Like the molds, they form a mycelium which is produced by true division (dichotomous branching of the individual threads), while, on the other hand, the thread formed from the germinal cell appears homogeneous and delicate in comparison with the double-contoured thread of the mold.

Perpetuation of the fungus occurs by segmentation of the air-hyphæ (see below) and subsequent dissemination.

In many of the streptothrixiæ there are formed at the ends or in the middle of the thread bulbous swellings, which are due to gelatinoid thickening of the membrane and are probably dependent upon regressive alterations. As a rule, these swellings are found only in the material taken from the animal body. They are found in the hard, *yellowish granules,* while in the gray and readily compressible granules only the nonbulbous (young) threads are seen. The chief representative of this group is:—

Actinomyces (Ray-fungus).

It is the frequent cause in cattle of tumors of the jaw, tongue, and oral cavity [wooden tongue, lumpy jaw]. It was first described by Bollinger in 1878. In man it also attacks by preference the *oral cavity,* especially carious teeth, and leads to woody-hard infiltration in the region of the angle of the jaw. It not infrequently enters the *respiratory*

passages, inducing fetid bronchitis, peribronchitic and pneumonic foci, as well as purulent and occasionally also serous pleuritis, peripleuritis, and mediastinal lesions. Sometimes the pathologic process in every way resembles the clinical picture of phthisis. More rarely it occurs as a purely local affection upon the *external skin* or in the abdominal cavity; in the latter case it may lead to ulceration, with rupture into the intestine and discharge of *actinomycotic pus in the stools.* J. Israel (who first accurately interpreted this mycosis in man), Ponfik, Bostroem, and others, have greatly contributed to the knowledge of the ray-fungus disease. J. Israel drew attention to carious teeth as the portal of entry, and in a case of pulmonary actinomycosis he found in an infected focus the fragment of a carious tooth. Bostroem, who accurately studied the biologic peculiarities of the fungus, believes that *grain, especially*

Fig. 39.—Actinomyces Hominis (Lung). × 350.

barley, upon which the fungus is frequently present, is the medium of infection. This harmonizes with the very frequent appearance of the affection during harvest months. [Actinomyces has been found upon young shoots of thorny shrubs and the bark of wood altered by moisture. Bollinger has detected it in milk, and Artant in eggs.—Brooks.]

When the disease exists, there will be found in the pus, discharged spontaneously or by incision, or in the purulent sputum or the purulent admixtures of the feces, *pale or deep yellow, scarcely visible, up to pinhead-sized granules,* usually of cheesy consistence. On crushing one of these granules there will often be seen, even in unstained preparations, numerous threads with

more or less glistening, pyriform or club-shaped ends, arranged in the shape of small fans or rounded radiate bodies (Fig. 39).

The diagnosis can usually be made with certainty even in unstained preparations. Now and then objects will be met with which greatly resemble these bodies, and which, on comparison with preparations of the true actinomyces, can be distinguished only by the fact that the club-shaped swellings are not so pronounced. Treatment of such preparations with alcohol or ether shows that the misleading objects are peculiar, radiately arranged *fat crystals.* The author has twice met such crystals: once in carcinomatous pleuritis and once in pulmonary abscess. The diagnosis is made *absolutely certain by staining.*

Staining.—1. Stain the dry preparation for from thirty to forty minutes in heated carbol fuchsin, then for from ten to fifteen minutes in Lugol's solution, decolor with alcohol, and wash in water.

2. Stain for from five to ten minutes in saturated anilin-water-gentian-violet solution, wash in physiologic salt solution, dry with filter-paper, place the preparation for two or three minutes in iodin-potassium iodid solution (1 or 2 to 100), dry again with filter-paper, decolor in xylol-anilin oil (1 to 2), and wash in xylol. Imbed in Canada balsam (Weigert). The mycelium appears stained dark blue. If it is desired also to stain the cellular elements, stain *first* for three minutes in lithium carmin, in order to secure bright-red contrast staining of the nuclei.

3. After Babès.—Stain for five minutes with saturated anilin-water-gentian-violet solution, then for twenty-four hours in a concentrated watery solution of safranin containing 2 per cent. of anilin-oil, then one minute in iodin-iodid potassium solution, and wash in alcohol. The threads of the fungi appear blue, the bulbous ends yellowish red.

4. By staining for a long time in a saturated solution of orcein in acetic acid and water Israel has succeeded in obtaining a Burgundy-red coloring of the terminal swellings.

The fungi described by Israel and Bostroem were at first considered identical. Further researches have shown, however, that there are *different species.* The Bostroem species, described by Kruse as streptothrix actinomyces, shows essentially *aërobic* growth and beautiful multiple branching reticulum, and is not transmissible to animals. The Wolf-Israel species, on the other hand, is chiefly anaërobic, of less active growth, and is pathogenic for animals. Finally, the description given by Bruns indicates that there are probably more than these two species of actinomyces.

For the preparation of cultures the characteristic granules are crushed between sterile glass plates and then freely spread upon the nutrient medium. After several days small, gray colonies develop and gradually grow to opaque nodules with radiate peripheral threads. Upon serum the colonies assume a reddish color and become covered with a whitish down (air-threads). After a time the colonies coalesce to form a firm, wrinkled deposit.

In conclusion, it may here be mentioned that branching and bulbous formations have recently been observed in different bacteria (tubercle bacillus, pseudotubercle bacillus, diphtheria bacillus, and glanders bacillus); so that there is a disposition on the part of some authorities to classify these bacteria with the streptothrixiæ.

3. BUDDING OR YEAST FUNGI [BLASTOMYCETES].

In contradistinction to the molds, which will presently be considered, the budding fungi possess neither mycelium nor conidia.

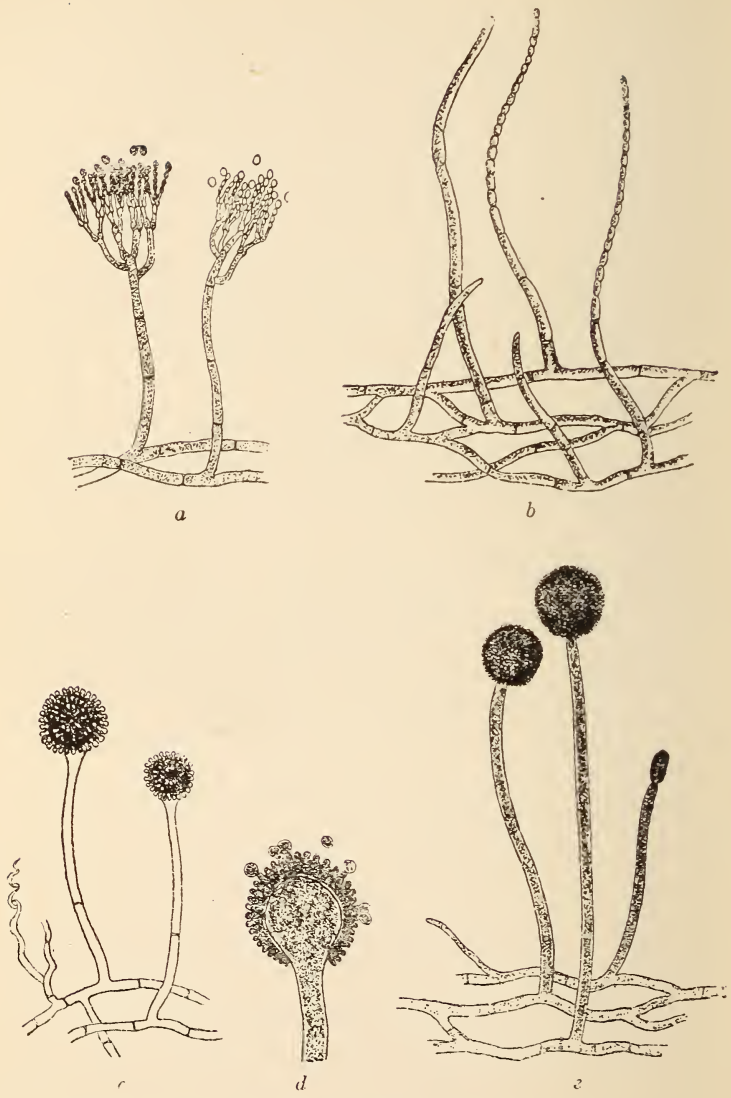
Multiplication occurs by simple budding. At one or several points upon the surface of the cell appear round or ovoid protuberances which more or less rapidly attain the size of the mother-cell and then separate or unite with others to form continuous chains or masses. The pellicle which forms upon the surface of spoiled alcoholic liquids consists of such masses of yeast-cells *(saccharomyces cerevisiæ)*, the most frequent form of budding fungi. In rare instances threads and mycelium may also occur in budding fungi.

In man *yeast fungi* occur most frequently in the vomited or siphoned *gastric contents* in *ectasia ventriculi* [dilatation of the stomach], in which condition great discomfort is caused by the fermentation they induce. Busse has shown that in rare instances yeasts may also be *pathogenic.* In a case of *chronic pyemia he found a yeast fungus which could be cultivated upon the ordinary media.* The cultivated fungus, when injected into animals, produced a disease similar to that observed in the human subject.

Whether the yeasts are of etiologic significance in malignant neoplasms is a question still wholly without proof.

4. MOLDS, OR THREAD FUNGI [HYPHOMYCETES].

These very widely distributed *fungi* are characterized by a simple arborescent structure (the so-called thallus), composed of achlorophyllous cells which have grown into more or less long, branched, and anastomosing filaments (hyphæ), forming a dense



[Fig. 40.—Molds. *(Baumgarten.)* (From Oertel, courtesy of P. Blakiston, Son & Co.)

a, Penicillium glaucum. *b*, Oïdium lactis. *c*, Aspergillus glaucus. *d*, The same more highly magnified. *e*, Mucor mucedo.]

reticulum *(mycelium)*. From the latter arise, during fructification, the *fruit-carriers* (fruit-hyphæ), which, by their peculiar structure or the kind of fruit they bear *(conidia* or *spores)*, permit differentiation of the various forms of molds.

Cultivation of the fungi is best accomplished upon *bread-pap*. Bread which has been slightly toasted at a moderate heat is rubbed to a fine powder and mixed in a glass flask with sufficient water to make a soft mush. The flask should then be sterilized in the steam sterilizer in the same manner as described for test-tubes (page 22).

Fig. 41.—Aspergillus Fumigatus. × 350.

The bulb-mold *(aspergillus)*, brush-mold *(penicillium)*, and *mucor* demand the attention of the physician. In the first (Fig. 41) the fruit-hyphæ show a terminal bulbous swelling upon which are seated more or less numerous, small, flask-shaped bodies, at the extremities of which the round or oval spores are found. In the *penicillium* the *segmented* fruit-bearers first divide into short branches, the so-called *basidia*, from which spring delicate, brushlike prolongations, the *sterygmata*, carrying at their ends a chain of spores. In the *mucors* (Fig. 42) the *nonseptate, nonbranching* fruit-hyphæ bear at their extremities a globular protoplasmic swelling, the *sporangium*, which incloses the spores in separate compartments, and is separated from the

fruit-carrier by a septum, the *columella*. When ripe, the sporangium bursts and the spores are liberated.

As a rule, molds but seldom give rise to pathologic alterations in the human body, for the reason that multiplication of the fungus can *never* take place in the living tissues. Consequently, the degree of disturbance produced is in direct proportion to the number of spores introduced. Apparently a number of the latter, even when they belong to a species with unquestionable pathogenic properties, can be received into the body without the appearance of any particular lesion.

Of the penicilliaceæ the *penicillium glaucum* may briefly be

Fig. 42.—Mucor Corymbifer. × 350.

mentioned. It is met with wherever molds are observed. It forms upon bread-paste at first as delicate, white flakes, which rapidly change to a greenish, mosslike structure. It possesses no pathogenic properties.

Pathogenic properties are known to be possessed by the *aspergillus fumigatus* (Fig. 41), which forms a dirty-green, flat growth and produces small, bright spores. It is observed in many forms of pneumonomycoses (Virchow, Dusch, and Lichtheim) and certain affections of the cornea (Leber), which are generated by trauma and the synchronous introduction of aspergillous vegetations. In pulmonary mycosis the fungus develops

as a saprophyte upon old tuberculous foci, hemorrhagic infarcts, cancer foci, etc. [According to Roger, aspergillus fumigatus may cause suppurative dermatitis, a special form of onychomycosis, suppurative keratitis, or lesions of the ear.—Brooks.]

The spores of this fungus are very common in bread. If nonsterilized bread is placed for several days in the incubator, a very luxuriant growth of aspergillus will generally be observed.

Some species of mucors may, according to Lichtheim, give rise to serious disturbances (ulcerations and lesions in the lungs and intestine). The *mucor corymbifer* (Fig. 42) is of especial significance. It forms a dense, snowy-white growth.

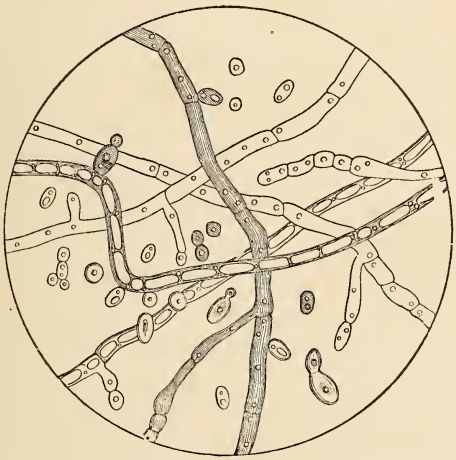


Fig. 43.—Soor [Thrush] Fungus. × 350.

Unstained preparations are best adapted for microscopic examination. They are prepared by teasing a portion of the mold mass in weakly ammoniated 50-per-cent. alcohol, and then examining in glycerin with 150 to 250 magnification. For *staining* it is advisable always to use Löffler's alkaline solution of methylene blue, which stains the mycelium and fruit-hyphæ, but not the spores.

The systematic classification of the species of *oïdium* is still unsettled. These fungi are of much simpler structure, without fruit-heads. On the contrary, the spores are formed by direct chainlike segmentation of the glassy-clear hyphæ which grow

out from the mycelium. The best-known species is *oïdium lactis,* which is constantly present in milk, especially that which has become sour.

The *thrush fungus (oïdium albicans)* (Fig. 43) was long confounded with the oïdium lactis. It is sometimes classed with the budding fungi [yeasts] (Grawitz), sometimes with the lower molds (Plaut). It is, however, quite justly classed with the molds. It occurs constantly in the white, flocculent or membranous deposits met with upon the mucous surface of the mouth in children or debilitated patients, especially consumptives [aphthæ, or mycotic or parasitic stomatitis]. It can always be removed without injury to the underlying mucous membrane. It is less frequently observed in the esophagus and in the vagina of pregnant women.

It almost never manifests *pathogenic properties.* E. Wagner, however, has observed the entrance of the fungus into the tissue of the esophageal mucous membrane, and Zenker [also Ribbert and Guidi] detected its presence in cerebral abscess [and by Schmorl and Klemperer in renal abscess and the lung respectively. According to Senator and Fritsch, oïdium may be found in the small and large intestine, bladder, anus, and vulva. —Brooks.] Quite recently, also, Klemperer succeeded in producing genuine general soor [thrush] mycosis in rabbits by intravenous injection of pure cultures.

It can be detected by crushing a small fragment of the deposit from the mucous membrane between a slide and a cover-glass. [Addition of liquor potassæ to the specimen renders it clearer.—Brooks.] When examined under the microscope such a preparation shows great numbers of glassy, branching, and jointed threads, with numerous free, brightly glistening spores, which also occur united to the threads.

Of far greater interest to the physician are a few fungi which also belong to the oïdium forms of fungi and which give rise to characteristic *diseases of the skin.* These are the *Achorion Schönleinii* of favus; the *trichophyton tonsurans,* which induces the same named form of *herpes;* and the *microsporon furfur* of *pityriasis versicolor.*

Achorion Schönleinii.

Favus occurs almost exclusively upon the hairy portions of the head, much more seldom upon the nails *(onychomycosis favosa)* or other parts of the body. In the beginning it appears as a yellow vesicle penetrated by a hair, or as a characteristic pure yellow scab *(scutulum)* surrounding a centrally located hair. By coalescence of numerous scutula extensive scabs are often produced, upon the outer zones of which distinct scutulum formation can usually be distinguished. The hairs always appear lusterless, are brittle, and fall out or can easily be removed by a slight pull; their root-sheaths are swollen, opaque, and yellow. The fungous growth occurring upon the nails leads either only

Fig. 44.—Achorion Schönleinii. × 400. (After *Bizzozero.*)

to circumscribed yellowish deposits or to a deeper affection of the nail itself, which loses its luster and becomes brittle.

The fungus, first discovered by Schönlein in 1839, and named after him, is found in the root-sheaths and between the fibers of the hair-trunk, as well as in the scrapings from the finger-nails, and especially *numerous*—often in pure culture—in the umbilicated, *yellow scutula.* For *examination* it is sufficient to rub a fragment of a favous flake with water or ammoniated alcohol and examine in glycerin. [The scales can readily be examined by moistening them in acetic acid or liquor potassæ, (preferably the latter) and then crushing between a cover-glass and slide.—Brooks.]

The fungus forms a *dense mycelium* composed of straight and wavy, branching, glassy threads showing here and there distinct notching or rows of large oval, *strongly refractive spores.* The latter also occur free in greater or lesser number, but are then usually arranged in chains and masses.

Trichophyton Tonsurans.

While the detection of the fungus of favus is always easy, the search for the fungus of *herpes tonsurans* is usually associated with great difficulty and always requires much patience. This is due to the fact that the fungous elements are never present in such numbers as in favus, and the inflammatory irritative phenomena are much more severe.

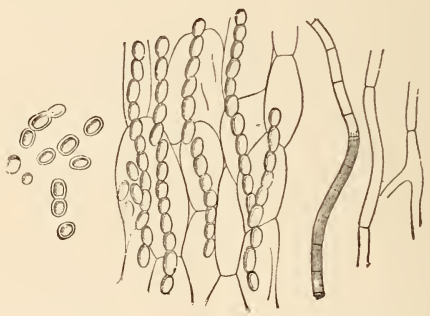


Fig. 45.—Trichophyton Tonsurans—Threads and Chains of Spores. × 400. (After *Bizzozero.*)

The fungous growth attacks *the hairy and nonhairy skin as well as the hair and nails.* The diseased epidermis is affected only in its superficial layers. The primary small, red, and often scaly spots which subsequently coalesce to form even hand-sized areas, are usually accompanied by vesicle and pustule formation. If, as is often the case, the *bearded part of the face* is attacked, intense inflammatory phenomena are usually present. In this locality as well as upon the *scalp,* where the inflammatory irritative phenomena are always less severe, the hairs at first become lusterless and brittle, and subsequently break off (hence *herpes tonsurans*), or the hairs may fall out as the result of the growth of the fungus into the root-sheaths or the hair-substance. The *finger-nails* also may become brittle in part or *in toto* from growth of the fungus.

The causative agent of herpes tonsurans, the *trichophyton tonsurans,* was discovered by Gruby and Malmsten in 1844-45. The often long, slightly branching threads form a distinct *myce-*

lium in which long, sporulated threads can usually be observed. Collections of free spores (as in favus) are rare, and in such instances their arrangement indicates their origin from spore-chains. On the other hand, large groups of spores are quite frequently seen in the root-sheaths and hairs.

Demonstration.—The examination of one or two diseased hairs seldom suffices for diagnosis; usually a large number—from ten to twelve or more—must be carefully inspected. It is best to examine the teased hair-stumps in glycerin to which a little acetic acid has been added.

Fig. 46.—Microsporon Furfur. × 350.

(Wax-paper drawing from a photomicrograph.)

Grawitz has positively proved by cultivation and successful inoculation that both of the fungi just described are different forms of oïdium.

Microsporon Furfur.

In 1848 Eichstedt discovered in *pityriasis versicolor* a fungus known as *microsporon furfur,* which is looked upon as the positive cause of this disease.

The fungi enter exclusively the uppermost layers of the epidermis, giving rise to the formation of small, usually circular, seldom somewhat elevated, pale-yellow or brownish spots. The eruptions, as a rule, spread slowly and often remain disseminated, but they not infrequently coalesce

and extend to such a degree that the whole trunk appears to be uniformly covered except for occasional smaller or larger islands of intervening healthy skin. The spots now and then present a delicate, branlike exfoliation.

Demonstration.—If one of the small spots is moistened for one-fourth minute with 10-per-cent. caustic potash solution and some of the softened upper layers scraped off with the edge of a scalpel and examined under the microscope with about 350 magnification, there will be seen numbers of chiefly short, bent, segmented, and branching, bright threads with the rounded, highly refractive spores arranged in grapelike masses.

Fig. 47.—Leptothrix *(l)* and Cercomonas *(c)*. × 350. From a Freshly Opened Tonsillar Abscess.

Appendix.

Leptothrix occurs most frequently as *leptothrix buccalis,* and was discovered by Leeuwenhoek. It very probably belongs to the *algæ*, not to the fission fungi, and is composed of dense bundles of straight, slender, transparent, nonbranching threads, which are inclosed in an extremely dense, finely granular mass. When examined with slight magnification, the individual threads present, in their interiors, small, round, regularly interrupted granules which *assume a blue color on addition of iodin.* This apparently indicates the presence of starch.

Leptothricial vegetations, which are constantly present in dental deposits and especially numerous in the cavities of decayed teeth, are concerned in the formation of tartar as well as decalcification (caries) of the teeth. Their demonstration is easy. With a toothpick or similar object take a small portion of the deposit on the teeth and place it upon a slide, with or without a drop of [so called] physiologic salt solution, and press upon it a cover-glass. If the iodin reaction be negative, acidulate the preparation with 2.5-per-cent. lactic acid.

The leptothrix was observed by Leyden and Jaffe in *pulmonary gangrene* and supposed by them to bear an etiologic relation to this process. Positive proof of this relation is lacking.

The author found a dense leptothricial flora in addition to cercomonas (see "Animal Parasites") in a freshly opened *tonsillar abscess* (Fig. 47). Whether in this case a pathogenic influence was attributable to the algæ or the infusoria or whether both only accidentally accompanied the suppuration could not be decided. The leptothrix, in addition to other flora of the oral cavity, was also found by the author in a freshly operated gangrenous pulmonary cavity.

(B) ANIMAL PARASITES.

Of the two great groups of *ecto-* and *ento- parasites,* the latter is of especial interest to the physician.

I. ECTOPARASITES.

Of the series of parasites living upon the surface of the body the following may be briefly mentioned. A few *fleas* and *ticks, pulex penetrans* (South America) and *ixodes ricinus,* cling for weeks to the body of man to suck his blood, and occasionally induce inflammation and ulceration. Another species of tick, *argas reflexus* (Fig. 48), the pigeon-tick, which sucks the blood of man only at *night,* and otherwise, as a rule, infesting pigeons only, has occasionally given rise to erysipelatoid eruption and severe general inflammatory edema of the external skin and mucous membrane, with distressing asthma (Alt).

This dirty-gray parasite is about 5 millimeters broad and 7 millimeters long, and is covered with a mosaiclike shell. It has four pairs of legs; in front of the first pair is the snout, in front of the second the genital aperture, and behind the fourth pair lies the cloaca. In the stage of fasting the tick is flattened; after sucking it is almost spheric and often increased to eight times its former weight.

Of the species of phthirius may be mentioned the *crab-louse (phthirius pubis),* which first and often exclusively infests the pubic hairs, but occasionally also migrates to all hairy parts of the body except the head. The female deposits her ova upon the hairs, to which the parasites very firmly attach themselves by hook-shaped claws (Fig. 49).

Acarus seu *demodex folliculorum,* the *hair-sac mite,* occurs at the bottom of almost every blackhead (comedo) and is readily demonstrated

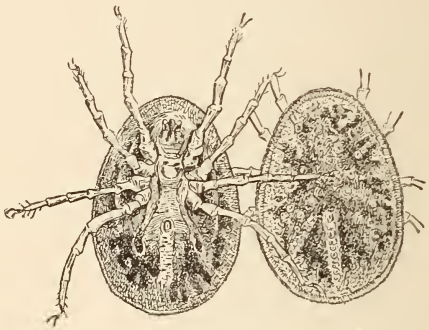


Fig. 48.—Argas reflexus. × 4. (After *Alt.*)

in smear preparations made with the sebaceous secretion. The mite is about 0.3 millimeter long. In addition to the head, it has a thorax, supplied with four pairs of feet, and a posterior body three or four times as long as the thorax. It is an insignificant parasite (Figs. 50 and 51).

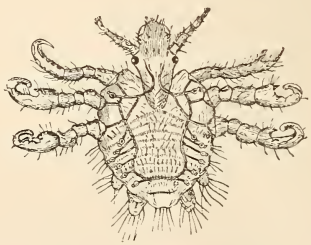


Fig. 49.—Phthirius Pubis [Crab-louse]. (After *Landois.*)

The *sarcoptes scabiei* causes the *itch,* or *scabies.* The female has suckers upon the two anterior pairs of legs and bristles upon the two posterior. The male is about one-third as large as the female, and has suckers also upon the two posterior pairs of legs. The female and its ova are best found in the *"mite channels."* To find it, the whole channel—the beginning of which is indicated by a small, usually dried vesicle, and the further course

by dark dots (dirt and excrement), and the end by a small, white point visible through the horny layer—should be removed with a small scalpel and crushed in dilute caustic potash between two glass slides. Besides the female, whose location is shown by the bright transparent point at the end of the channel, there will be seen a number of more or less developed ova showing granular contents or almost ripe embryos (Fig. 52).

II. ENTOPARASITES.

1. Protozoa.

Of these, the **malarial plasmodia** (Plate II, Fig. 11) are of chief interest. The organisms, discovered in 1882 by Laveran

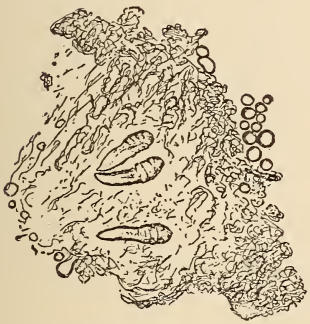


Fig. 50.—Acarus Folliculorum with Low Magnification.

Fig. 51.—The Same with High Magnification.

and Richard and accurately studied by Marchiafava and Celli, are of extreme clinical importance. Their specific etiologic significance in malaria can no longer be doubted, and their discovery permits the surmise that many other infectious diseases also are produced, not by bacteria, but by similar *protoplasmic bodies* standing on the borderline of the animal and vegetable world. Their systematic classification is not yet settled. According to Metchnikoff, they are very closely related to the coccidia, to which view R. Koch is, with certain limitations, also inclined. For the present, however, it is well to consider the malarial

plasmodia as a special group, of which the following species are known:—

1. *The parasite of quartan fever* (Golgi).
2. *The parasite of tertian fever* (Golgi).
3. *The parasite of tropical fever—estivo-autumnal fever of the Italians* (Marchiafava).
4. *The malarialoid parasite of monkeys* (R. Koch).
5. *Proteosoma Grassi* (Labbé).
6. *Halteridium Danilewskyi* (Labbé).

The parasites classed under Nos. 4, 5, and 6, which occur in monkeys and birds, must here be considered, because in them the whole process of development of the parasites has been successfully studied, and their close relation to the malarial plasmodia

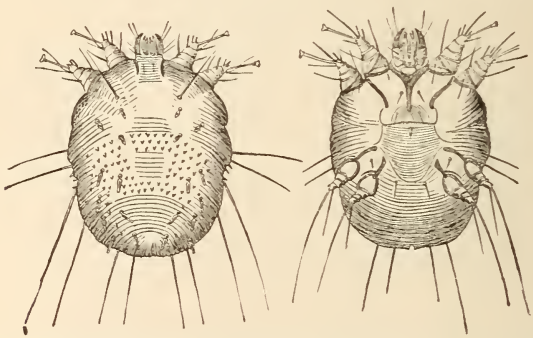


Fig. 52.—Sarcoptes Scabiei. Female seen from Above and Below. (After *Gudden.*)

of man has permitted conclusions as to the biologic characters of the latter. The following has been determined by the researches of MacCallum, Ross, and R. Koch in reference to halteridium and proteosoma:—

The proteosoma occurs in various birds (sparrows, etc.) inhabiting warm climates. It appears in the blood of these animals in the form of smaller or larger plasma granules within the red blood-corpuscles, and is readily recognizable, on staining with Romanowsky's stain (see below), by the *ruby-red chromatin nucleus and the blue-stained protoplasma.* With gradual growth of the "rings" fine pigment granules are deposited in them. Then division of the parasite into 4, 8, or 16 parts takes place, all of which consist of a small chromatin nucleus and protoplasm. The young forms again enter red blood-corpuscles, and the cycle is repeated anew.

In addition to this mode of development, which was designated by R. Koch as "*endogenous,*" a second—"*exogenous*"—has been determined. In some of the parasites *small, threadlike bodies, spermatozoa,* are seen to escape from the chromatin nucleus. These bodies exhibit active vibratile independent motility, by means of which they enter another plasmodium. With this occurrence development within the primary host is, as far as is now known, completed. Further growth takes place in an *intermediate host.* The intermediate host has been demonstrated to be a particular *species of mosquito.* If the latter has sucked blood from birds sick with malaria after from twelve to fifteen hours a new "*vermicular*" form can be seen on microscopic examination to develop from the parasites last observed in the bird's blood. At first there protrudes from the spheric plasmodium a process which steadily grows larger and assumes the form of a vermicule. This escapes from the envelope, and after forty-eight hours these new forms have disappeared. In their place appear, upon the *external wall of the stomach,* spheric, pigmented, *conidialike* corpuscles, which, in the course of six or seven days, are transformed into numerous *sickle spores.* These then rapidly penetrate to the *poison* [*or salivary*] *glands of the mosquito,* whence they pass into the animal body when the mosquito bites a bird.

This mode of infection of birds through mosquitoes has been positively demonstrated by Ross and R. Koch. A similar process is observed with *halteridium* [common in crows]. The young parasite has a dumb-bell-shaped form, which soon changes to a spheroid. The blood-corpuscle is destroyed and the parasite set free.

With Romanowsky's stain it is possible to distinguish two varieties of plasmodia. One is characterized by a large, compact chromatin nucleus, the other by a small nucleus evenly surrounded by intensely-blue-stained protoplasm. Out of the first plasmodia (the male individuals)—*i.e.,* from the chromatin nucleus—a number of cilialike bodies develop which are endowed with active independent motility and soon separate themselves from the body. These spermatozoa fructify the second form of plasmodia (the female individuals), which they penetrate. After copulation the female spheric parasite sends out a curved, hornlike process, which, as in the proteosoma, develops into a vermicule and finally becomes separated from the spherule. The pigment is retained in the vermicule.

When treated with Romanowsky's stain, the "vermicules" show a ruby-red chromatin nucleus, blue-stained protoplasm, and in this a few round, unstained spots.

Nothing is at present known of the further development of halteridium. According to the analogy of proteosoma, it may be assumed that an intermediate host is required.

In the *malarial plasmodia of man, also, endogenous and exogenous development* must be distinguished. The *first* has

L. of C.

been quite well investigated; the second is but little elucidated. It is certain, however, that the *intermediate host is a species of mosquito.*

The *fundamental type of the malarial parasites is the ring form,* in which a distinct nucleus can often be distinguished. The size of the ring varies in the different forms; in all it is at first small and increases with the development of the parasite; it varies between 1 and 10 μ. The germs lie upon or within the red blood-corpuscles. In *unstained* preparations *distinct amœboid movement* can be seen. As a rule, but *one* parasite lies in a blood-cell, though two, three, and more sometimes occupy a single corpuscle.

As growth advances a dustlike pigment, which is derived from digested hemoglobin, makes its appearance at the periphery of the ring. The pigment granules are often observed to manifest active dancing movements within the parasite, which action Mannaberg attributes to a flowing movement of the plasma.

The final stage of development of the parasites is *sporulation.* In the fully developed rings distinct segmentation of the original body into from 4 to 8, 12, or 20 parts ("spores") can be seen. These are liberated by bursting of the envelope, and the above-described process of development is repeated, each spore entering a red blood-corpuscle to grow into a ring.

In *unstained* preparations the recognition of the forms just described requires great experience. On the other hand, *stained* preparations not only facilitate the finding of the parasites, but also enable the examiner to distinguish structural differences.

For *diagnosis,* simple staining of dried blood preparations with Chenzinsky's solution suffices.

Much sharper pictures can be secured by the methods of Romanowsky. The latter found that, under certain circumstances, *bright-red* nuclei could be brought out in the parasites with pure watery eosin-methylene blue solution. This occurred, however, only when the dyes were freshly mixed, and when the *methylene blue solution had been altered by long standing and the presence of molds.* Nocht improved the method upon the basis of his observation that the alteration of the watery methylene blue solution can be secured by heating for two days at 66° C., whereby the solution assumes a *violet* hue.

My former assistant, Dr. Reuter, has greatly simplified the procedure by a convenient preparation of the so-called A-methylene blue (Grübler's), which stains the chromatin. It is employed dissolved in methyl [wood] alcohol. Reuter's method is as follows: The air-dried smear preparations are fixed by pouring upon them formol-alcohol (formol, 10 parts; absolute alcohol, 90 parts) and at once carefully drying with filter-paper. (The procedure takes about three seconds and gives the best results for all methods of blood-staining.) They are then placed in a spacious dish (cover of a Petri dish) and covered with the staining solution (distilled water, 20 cubic centimeters; A-methylene blue-eosin solution,—Grübler,—30 drops) mixed in a graduate. By agitating the dish as in developing a photographic plate, precipitation of the dye and staining of the preparations can be hastened. In all instances the process is completed in from fifteen to thirty minutes. Finally, wash in flowing distilled water, remove excess of water with filter-paper, dry in the air [or high above the flame of an alcohol-lamp or Bunsen burner—Brooks], and examine in immersion oil without cover-glass or after mounting in Canada balsam. This method has given us entire satisfaction.

Another sure and convenient method is that of Giemsa, of Hamburg. It is as follows: According to requirements, prepare an 0.8 per 1000 watery solution of a dye known as azur 2 (methylene-azur-chlorhydrate + methylene blue, medicinal, Höchst); also a large quantity of a 0.05 per 1000 watery solution of eosin (5 cubic centimeters of a 1-per-cent. eosin solution in 1000 cubic centimeters of water). Distilled water should be used for the solutions, which should be kept ready in a dark vessel. In preparing the staining mixture 10 cubic centimeters of the eosin solution should each time be placed in a graduate and 1 cubic centimeter of the azur solution added and the solution shaken. Stain for from fifteen to thirty minutes. Wash for from five to ten seconds in a strong stream of water, mount in nonacid balsam, and examine in immersion oil.

The *bright-red chromatin granules* are then distinctly seen. If the red coloration of the red blood-corpuscles is obscured by a bluish tinge, it is advisable subsequently to stain for a short time with eosin solution.

In regard to the *red granules,* it may now with *certainty be assumed that they represent an essential constituent of the parasite,* as is shown by the fact that each of the spores possesses a delicate chromatin nucleus.

The chromatin nucleus usually lies at the surface of the rings, less often in the interior; it is irregular in form, not infrequently ring-shaped, and varies in size and depth of staining.

Besides the chromatin, a round, colorless spot *(vacuole)* is sometimes observed in the ring-formed plasmodia.

It is probable that, in addition to *sporulation,* a second mode of propagation occurs. The observation that very actively motile flagella which are gradually set free are seen to develop from many parasites speaks in favor of this view. As to the further fate of these flagella nothing is positively known, but the analogy with the above-described processes observed in proteosoma and halteridium is so unmistakable that it may be surmised that a similar course is followed here.

Thus far the following malarial plasmodia have been accurately studied with the aid of the methods already described:—

1. The Parasite of Quartan Fever.—As Golgi first determined, it completes its development in seventy-two hours, and first appears as a small, nonpigmented corpuscle located either upon or within a red blood-corpuscle. After twenty-four hours it has become larger and deposited pigment at its periphery; the *primarily* somewhat *sluggish ameboid movements* cease. After sixty hours the parasite almost fills the red blood-corpuscle and the process of sporulation begins, the pigment collecting in the center and a radiate segmentation making its appearance in "daisy-flower" form. The plasmodium then divides into ten parts (spores), which become free by bursting the capsule, and begin the cycle anew.

2. The Parasite of Tertian Fever.—It develops in forty-eight hours, and begins, as in quartan, as a delicate, *actively motile* body within a red blood-corpuscle. On further growth the parasite produces very irregular ring forms which sometimes appear long or oval, sometimes supplied with prolongations. Here also pigment becomes visible with the loss of color of the affected red blood-corpuscle, and is usually irregularly distributed. Later on the ring form becomes indistinct and the

blue-stained marginal zone shows very irregular contour; so that scarcely one plasmodium resembles another. The red chromatin granules also occur here.

The parasites often fill the whole blood-cell; sometimes the latter seems even to be enlarged thereby.

Sporulation takes place as follows: The pigment usually collects in a heap in the center of the parasite, and the body of the latter divides into from 15 to 20 spores. The plasmodium then presents a certain resemblance to a mulberry. The young forms disseminated after bursting of the envelope take up dyes very readily, and when treated with the Romanowsky-Nocht stain the chromatin nucleus is beautifully shown. Sporulation forms are found chiefly *at the time of fever.* The author has, however, repeatedly met with the parasites in the various stages of development *at the same time* without the fever manifesting any deviation in its course.

In *fresh* preparations flagella can readily be distinguished in the tertian form.

As Golgi first demonstrated, the occurrence of *febris quotidiana* can be explained by the daily maturing of *two* generations of tertian parasites or of *three* generations of quartan plasmodia.

Strange to relate, isolated cases are met with in which all stages of development are sometimes seen together, and the fever nevertheless pursues a regular tertian type.

3. The Parasite of Tropical Fever.—In addition to the ring form this parasite shows, chief of all, the peculiar *crescent form* (Laveran) which is characteristic of it. The ring is delicate and thin and provided with a small knob (chromatin nucleus). On further development the rings resemble those of tertain type; the large pigmented forms, however, are wanting (the fever-curve also provides against confusion). In the final stadium of the ring there appears at a point opposite the nucleus a crescentlike swelling which stains blue, and now and then contains some dustlike pigment, and occasionally two or three colorless points (vacuoles?). In sporulation, divison into 10 or 12 parts occurs. If the fever has existed for several days, there will be seen in the blood the peculiar "crescents" which R. Koch associates with exogenous development. In *fresh* preparations the crescents appear as sausage-shaped, bent bodies, in the centers of

each of which is located a wreath of pigment, which, like the protoplasm, manifests active motility. The development of flagella can also be seen. On staining, the poles and marginal portions of the parasites readily take up the dye. Chromatin staining is seldom distinct.

The crescents also occur within the red blood-corpuscle, but the latter is usually so far destroyed that often only a small edge of it remains. Apparently they represent *lasting forms,* since they are often still demonstrable *after* the attack in the *afebrile period.* In such cases a recidive is usually later observed.

Fig. 53.—Amœba Coli. From Dysenteric Stool. Zeiss, I; oil immersion, $^1/_{12}$.

Amœba Coli (Lösch).

The amœba coli likewise belongs to the rhizopoda. It appears as a motile mass of protoplasm 0.02 to 0.035 millimeter in size, which inhabits chiefly the human colon. It is almost constantly present in typhlitic fecal abscesses. In the moving amœba there can usually be seen, in addition to a delicate nucleus and nucleolus, one, two, or more bright spots ("vacuoles") about the size of the nucleus. Its pathogenicity has not yet been positively established. It is very probable, however, that it is an etiologic factor in many *forms of dysentery* (see section on "Intestinal Affections").

Kartulis and many others do not doubt the specificity of the amœba. In examining dysenteric dejecta I have frequently seen

amœbæ in such large numbers as to lead me to ascribe an etiologic rôle to these microbes. On the other hand, however, the objection that they are only harmless inhabitants of the intestine cannot with sufficient reasons be denied, especially in view of the bacterial findings described on page 80, the causative significance of which has been so strongly confirmed by the Widal reaction. [According to Roger, Marchoux succeeded in producing serious dysentery and hepatic abscesses in cats by introducing dysenteric dejecta into their digestive organs. These symptoms do not appear when the same dejecta are employed after subjecting them to a temperature which kills the amœbæ without affecting the accompanying bacteria.—Brooks.]

It is necessary to examine quite *fresh discharges.* Under such circumstances with a little care ameboid movements of the cells will be seen. Aside from the slow progression movement and protrusion of pseudopodia, a ringlike constriction of the cell is not infrequently observed, through which the contents of one-half of the cell is squeezed into the other.

Gregarinæ.

The views in reference to many pathogenic effects attributed to the sporozoa belonging to the protozoa are still so little elucidated that only a few words will be devoted to their consideration. The best known are the "*gregarinæ,*" which appear as spheric or oval, slightly refractive bodies 1.5 to 2.5 μ in size, rarely without nucleus and capsule. They occasionally occur in the liver, and, among others, they are accredited with the production of *molluscum contagiosum.* Whether they take part in the production of the polymyositis described by E. Wagner and others has not yet been settled.

Cercomonas and Trichomonas.[1]

Thus far other lower organisms belonging to the class of *infusoria* and which are in part separated under the name *flagellata,* possess but a secondary interest for the physician. They are spheric or ovoid, unicellular organisms, which, in addition to a short, thin caudal thread, possess one or more delicate flagella by means of which the infusorium is set in active motion. The forms provided with but *one* flagellum are named **cercomonas**; the complicated forms, **trichomonas.** They thrive best in the mucous secretion of the vagina and intestine, but they are also

[1] See Fig. 47.

found in the nose, without giving rise to any pathologic manifestations.

Kannenberg described their occurrence in pulmonary gangrene. The author found them in a freshly opened *tonsillar abscess* and also in the sputum of a patient presenting the features of multiple *pulmonary gangrene.* In the latter case he could also find them in the *fresh pus taken at necropsy from a small focus*—a proof that they did not primarily enter the sputum from the open air-passages or the mouth. That they exert pathogenic effects is very questionable; at least the author would not attribute to them an etiologic rôle in these two cases, for, in addition to the cercomonas, numerous cocci, which in every way resembled the flora of the oral cavity, were also present.

It is of further clinical interest to note that these organisms have also been observed in the fresh urine of men (Marchand, Miura). In both instances the patients were elderly individuals. In Miura's case there was good reason to believe that the organisms occupied the urethra.

The author found these flagellated bodies to be particularly numerous in the bright, dirty-yellow-colored flakes, varying in size from minute specks to that of a milletseed or bean. They could be examined at once in fresh crush preparations. The ovoid bodies were between 6 and 12 μ broad and usually 12 μ long; the flagella were about one and one-half to two and one-half times as long as the whole body of the organism.

The protoplasm is either perfectly homogeneous or, what is most frequently the case, filled with granules and small vacuoles. An oral aperture cannot be demonstrated. Sometimes a distinct undulating (serrated) seam is observed along one side of the body.

The flagellum is employed either for twisting, revolving movements of the cell or for fixation; apparently also for entrapping. When the infusorium has fixed itself by aid of the flagellum the cell-body often performs the liveliest revolutions. The cell then appears to be somewhat spheric and with the concentrically curled, motile flagellum upon its surface. In the author's case of tonsillar abscess the bright clumps of protoplasm observed in the pus underwent many changes of form during their migrations between the dense reticulum formed by *leptothricial threads.*

Staining of the objects is unnecessary and difficult. Kannenberg gives the following *directions:* The thinly spread material is rubbed up with about 1-per-cent. solution of sodium chloride. A drop of this is freely spread upon a cover-glass or slide and dried. Stain with watery solution of methyl-violet, wash in water, and treat the undried preparation with concentrated acetic acid-potassium solution. The protoplasm body shows distinct blue coloration.

Marchand recommends staining with weak methylene blue solution after previous treatment with acetic acid and then placing in concentrated sublimate solution in order to bring out the flagella and caudal appendage.

In many cases of *scorbutus,* and especially in *acute pernicious* anemia, Klebs attributes special significance to a smaller form of cercomonas. Klebs found them as spheric or slightly oval, glistening bodies about from 1 to 2.8 μ in size, which manifested active fluttering movements by the aid of one or two flagella. They presented no distinct structural peculiarities. Since "numbers" of them occurred in the blood in many cases of acute pernicious anemia, Klebs believed he was justified in designating them as the causative agents. Thus far only Frankenhäuser and Neelsen have recorded similar observations. Klebs also observed these organisms in the blood in severe cases of scorbutus accompanied by multiple disseminated hemorrhages.

Another species of infusoria, *megastoma entericum,* 15 to 18 μ long and 8 to 12 μ broad, is occasionally met with in *diarrheal* stools, especially in the gelatinoid mucous dejecta observed in children. It is pear-shaped, with a gradually tapering posterior part, and as organs of locomotion possesses four pairs of delicate flagella which are visible only after treating it with 10-per-cent. soda solution and the use of the oil-immersion lens. Examined upon the warm stage the organism can be seen in active motion. Motility ceases on cooling as well as on heating the preparation above 50° C. The organisms soon die outside of the body. They usually appear in the stools "encysted" as delicate, ovoid eggs surrounded by a distinct envelope. These ova measure about 10 to 13 μ in length and 8 to 9 μ in width (Moritz). Pathogenic properties are not known. They have occasionally been found in large numbers in the stools of perfectly healthy individuals with faultless digestion.

2. Intestinal Worms.

Under this heading are distinguished parasites observed especially in the intestine and its dejecta:—

(a) Roundworms, **nematodes** (νῆμα = thread [εἶδος, form]).
(b) Tapeworms, **cestodes** (κεστός = a girdle [εἶδος, form]).
(c) Sucking worms, **trematodes** (τρῆμα = hole, sucker [εἶδος, form]).

While the young forms of the previously described parasites are capable of acting injuriously upon the host at once and on

the spot, the embryos of the intestinal worms cannot do so. On the contrary, *they must first accomplish more or less peculiar migrations.* These are quite simple in the case of the trichinæ, the embryos of which do not desert the body of the host, but only wander or are carried into other organs, as well as in the case of oxyuris, the ova of which must re-enter the intestine of the host (but always *per os*) in order again to produce living embryos. The process is more complicated in ascaris lumbricoides, the eggs of which must first remain for some time in moist earth before they enter the intestine. Similar conditions exist in the case of anchylostomum and trichocephalus, and they are much more complex in filaria.

(a) Nematodes.

These worms are cylindric, slender, and nonsegmented. The oral aperture, which is always located at the end of the body, is provided with soft, but somewhat firm, lips. The straight intestine, which is divided into pharynx and chylgaster, rarely opens at the posterior extremity of the body, but usually somewhat in front of this upon the ventral surface. The two to four lines noticeable upon the larger forms mark the course of the excretory organs and nerves. The males usually present a coiled caudal extremity and a common ano-genital opening (cloaca). The females are usually quite numerous and larger. The vulva is placed at about the middle of the ventral surface. The ova are very resistant and surrounded by a transparent, firm chitinous or lime-shell which is sometimes covered with a rough, stained albuminous envelope (ascaris). Occasionally small plugs are present at the poles (nutriment?). The process of development is direct; the embryos can be plainly recognized as round worms.

Of the nematodes the following may be mentioned:—

Anguillula intestinalis occurs in the small intestine and lives upon the chyme, and not upon the blood. It was formerly looked upon as the cause of Cochin-China diarrhea, but alone it appears to be incapable of producing injurious effects. It is important to note that it often occurs in conjunction with anchylostomum.

Oxyuris Vermicularis.—The *male* is 4 millimeters long and has a blunt caudal extremity (Fig. 54). The *female* measures about 10 millimeters in length and is provided with an awl-shaped tail. Upon the cephalic end there are three small lips. The male possesses a rod-shaped spiculum (firm copulative organ). The ova are 0.05 millimeter long and about half as broad (Fig. 56, *b*). The *embryo* is fully developed when the ova

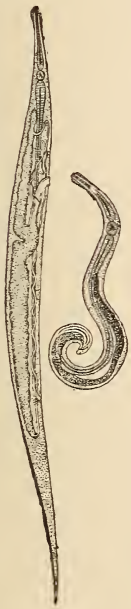


Fig. 54.—Oxyuris Vermicularis. Female and Male. (After *Leuckart.*)

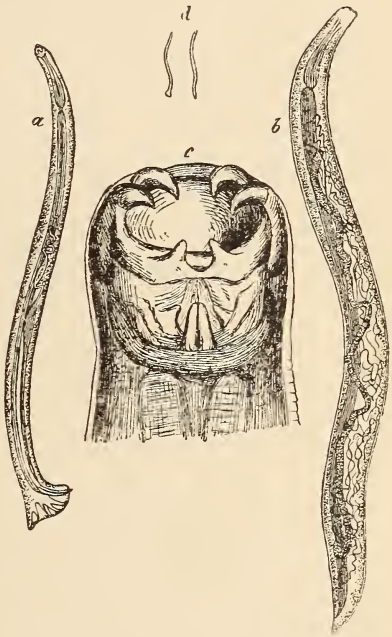


Fig. 55.—Anchylostomum Duodenale. (After *Leuckart.*)

a, Male. *b*, Female. *c*, Head. *d*, Natural size.

are deposited. The worms live upon the feces in the large intestine and *wander out through the anus at evening and night. The ova gain entrance to the mouth from soiled fingers, and are subsequently freed from their envelopes by the gastric juice. The liberated embryos then migrate to the colon.*

An examination for oxyuris and its ova is demanded in pruritus ani et vulvæ, irritative conditions of the genital region, etc.

Anchylostomum Duodenale, s. Strongylus (s. Dochmius) Duodenalis.[1]

The strongylidæ present at the anterior end of the body a bellied mouth-capsule with jawlike thickenings, four strong, clawlike hooks, and two comparatively delicate teeth. The tail of the *male* terminates in the bursa copulatrix, a quite broad trilobed pocket, peculiar to the strongylidæ, at the bottom of which open the intestine and vas deferens, accompanied by two long spicula. The vulva of the female lies behind the middle of the body.

The *male* attains a length of 10 millimeters; the *female,* 18 millimeters. The ova (Fig. 56, *d*) are 0.023 millimeter broad and 0.044 millimeter long.

Fig. 56.—Eggs of Nematodes. (After *Leuckart.*)

a, Ascaris. *b*, Oxyuris. *c*, Trichocephalus. *d*, Anchylostomum. *e*, Bothriocephalus. *f*, Tænia saginata. *g*, Tænia solium.

These parasites inhabit the upper portions of the small intestine, where, through the agency of their armed mouth, they *suck the blood, producing dangerous and fatal anemia.* They were discovered by Griesinger in 1851 to be the cause of Egyptian chlorosis. They are widely distributed in the tropics as well as in Italy, and since the construction of the St. Gothard tunnel they have gained entrance into Germany (Würzburg). They were brought to Aix-la-Chapelle and Cologne by Walloonic laborers in brick-clay, which, as is well known, is worked in a moist state. Their morphologic and biologic characteristics, as well as their relation to many forms of severe chronic anemia, have been investigated by Perroncito, Bizzozero, Bäumler, Sahli, Mayer, Leichtenstern, and others.

The ova are found in large numbers in the feces, and require

[1] See Fig. 55.

water and moist earth for their further development. The young worms escape from the eggshell and creep around everywhere, get upon the hands of earth- (clay-) workers and thence to the mouth, or they are ingested with drinking-water. The parasites were found by Bäumler in the intestine two years after infection and by Perroncito even four years after.

Trichocephalus Dispar.

The *male* is from 40 to 45 millimeters long; the *female* up to 50 millimeters in length. The *ova* are 0.05 to 0.054 millimeter (Fig. 56, *e*) in size. The anterior portion of the body is threadlike; the shorter posterior portion swollen. The female genital aperture lies at the junction of both. The male has a spiculum 2.5 millimeters long. The worm is usually found in the *cecum* in numbers varying from four to twelve. The threadlike anterior end is buried in the mucous membrane. When present in large numbers, violent reflex *cerebral phenomena* are observed.

Trichina Spiralis.

Infection of man takes place through the ingestion of trichinous pork which is eaten raw or underdone. The capsules of the *"muscle trichinæ"* are dissolved by the gastric juice. The liberated trichinæ develop within two or three days into "sexually ripe" forms, which copulate and, during a sojourn of about five weeks in the intestine, bring forth *enormous numbers* of young trichinæ. The mature worms are of hairlike form, with a somewhat thickened, round body-end. The *male* is 1.5 millimeters and the *female* up to 3 millimeters long. The pharyngeal portion of the intestine is greatly developed, and in the male occupies about two-thirds the length of the body. The anus is located at the posterior extremity of the body. Upon the caudal extremity of the male two conic papillæ can be seen, and between these four smaller ones. Spiculum is absent. The vulva is located in the anterior third of the body.

The embryos penetrate the intestinal wall very soon after birth[1] and migrate to the various parts of the body, where, by

[1] According to the researches of Askanazy, it is quite probable that the *intestinal trichinæ* penetrate the mucous membrane of the intestine and there (or in the *chyle vessels*) deposit their young. The lymph-stream then conveys the embryos onward.

settlement in the muscles, they cause the phenomena of the often fatal *"trichinosis,"* the general manifestations of which cannot be discussed in this place. The parts of the musculature most severely affected are the diaphragmatic, abdominal, cervical, laryngeal, facial, and ocular muscles, all of which are more or less disturbed in function. The most numerous collections are found in the neighborhood of tendinous attachments. After about fourteen days the young trichinæ develop within the primitive

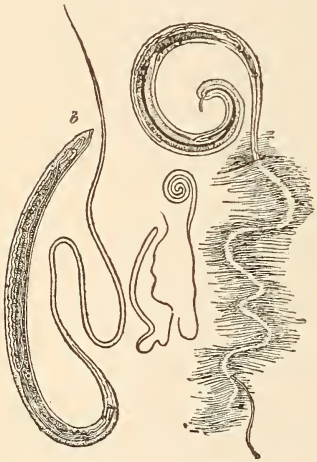


Fig. 57.—Trichocephalus Dispar. (After *Leuckart.*)

a, Male. *b*, Female. Both in natural size and highly magnified.

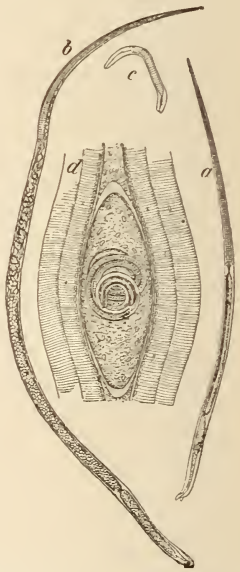


Fig. 58.—Trichina Spiralis. (After *Claus.*)

a, Male. *b*, Female. *c*, Embryo. *d*, Muscle trichina.

muscle bundles to mature *"muscle trichinæ,"* which coil themselves up in spiral form. Within the next two or three weeks degenerative and inflammatory disturbances occur in the surrounding muscle-tissue, as the result of which a spindle-shaped capsule is formed around the muscle trichina. Encapsulation is generally completed at the end of the fifth week after invasion. In the next succeeding months distinct thickening and gradual *calcification* occur, the latter beginning at the poles of the

spindle-formed swellings and slowly advancing toward the center, whereby the long axis of the cyst lies in the direction of the muscle fibers. The encapsulated trichinæ remain capable of development for years (eleven and more). The calcified capsule is 0.3 to 0.4 millimeter long and just visible to the naked eye; within this but one trichina is found as a rule; occasionally, however, three or four are present.

For our knowledge of this process we are indebted to the researches of Zenker, Leuckart, and Virchow.

The diagnosis of *trichinosis* is assured by the detection in the feces of sexually mature worms and of the small free embryos which have failed to penetrate the intestinal wall (see above), as well as by the examination of fragments of muscle. For this purpose either a piece of the painful and swollen muscles of the neck is removed with a harpoon or the muscle is exposed by incision and flat portions removed in the direction of the long axis of the muscle-fibers with curved scissors. These fragments are then thoroughly teased, cleared with glycerin-acetic acid and examined with a magnification of 80 to 100 diameters.

In the *obligatory trichina inspection* as conducted in Germany, it is the rule to remove a number (six to eight) of muscle samples from the freshly slaughtered swine. The various provincial regulations differ in regard to the number of samples as well as to the muscles to be examined. In every case it is necessary that a number of samples be taken from *both halves* of the body of the animal, especially from the muscular parts of the diaphragm, the abdominal and masticatory muscles (or eye and tongue muscles); a sample is then also to be taken from the laryngeal and intercostal muscles. The pieces of muscle removed with curved scissors should be about 5 to 6 centimeters long and 2.5 centimeters broad; from these are then made transparent preparations about 0.5 centimeter broad and 1 centimeter long, which are to be well teased in water and carefully examined with a magnification of 80 to 100 diameters.

Ascaris Lumbricoides.

The *male* is up to 250 millimeters long and 3.2 millimeters thick; the *female,* up to 400 millimeters long and 5 millimeters

thick. The worm is cylindric, tapering at both extremities, and has three lips upon the cephalic end. The vulva is located in the middle third of the body; the anus at the posterior extremity. The male has two club-shaped spicula. The worms inhabit chiefly the *small intestine,* and are very frequent in children and the insane. The ova are 0.05 to 0.06 millimeter in diameter. They are discharged in large numbers with the feces. *Infection of man* occurs when the eggs have remained about from four to six weeks in moist surroundings, where the embryo develops and the nodular, brownish external shell is not lost (as is possible in water). The embryo also develops within the egg in which the external shell is destroyed, but it is then killed in the stomach by the action of the gastric juice. Protected by the external shell it passes (without the agency of an intermediate

Fig. 59.—Filaria Embryos. (After *von Jaksch.*)

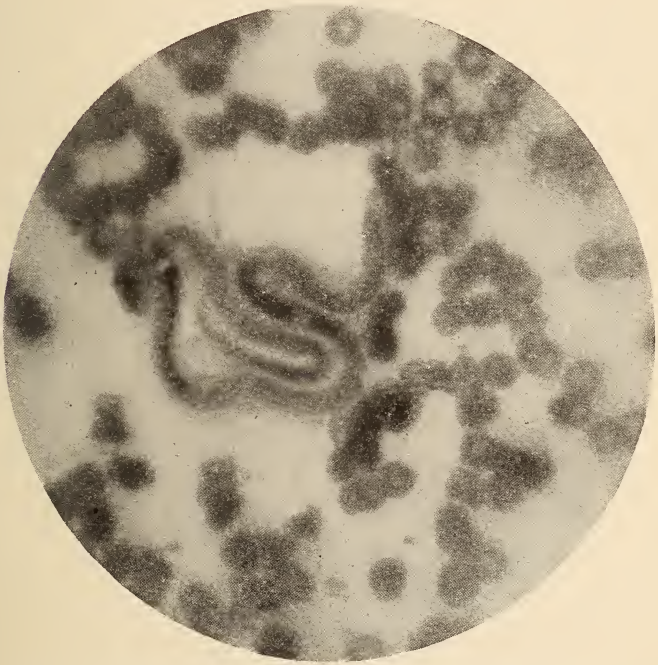
host) through the stomach to the small intestine, where it bores through the shell by means of an embryonal spicule (Lutz). If the embryo is not developed, the ova which have reached the intestine by the mouth are discharged from the body undestroyed.

Filaria Bancroftii—Filaria Sanguinis Hominis.

The mature, 10 centimeters long filiform worm is located in the subcutaneous cellular tissue of the scrotum or legs, where it induces intense swelling, especially of the glands. *Clinically, the embryos* (Fig. 59) *almost exclusively are of interest, because they circulate in the blood and in addition to elimination with other secretions are discharged especially with the urine* and produce the characteristic *chyluria* and *hematuria* (which see, page 303). They are delicate, transparent, cylindric organisms with rounded head and tapering tail. A structureless sheath projects beyond both extremities, sometimes in the form of a flagellum, sometimes as a hood, within which the embryo usually moves quite actively. *According to Scheube,* they are 0.2 millimeter long and 0.004 millimeter thick. They are said to be found in the blood at night,

and in the daytime only when the patient sleeps during this period. According to the researches of Manson, this phenomenon appears to depend upon the width of the capillaries, the diameter of which is usually greater at night. The author, however, was able to find the parasites during the day even in the absence of the above-mentioned proviso.

[Fig. 60 shows a filarial embryo in blood obtained from a male patient during the day. The case occurred in the practice of Dr. C. R. L.



[Fig. 60.—Filaria Sanguinis Hominis in Human Blood. From Photomicrograph. × 500.]

Putnam, of New York City, and the specimen from which the photograph was taken was presented to me by him.—Brooks.]

The migration of the embryos is very peculiar. They enter the intestine of the mosquito during the act of blood-sucking and on death of the mosquito, which occurs soon after the deposition of its ova, they gain access to water. Whether they now enter a water animal as host or immediately pass into the human stomach with the drink is still an unsettled question.

(b) Cestodes.

Common Characteristics and Attributes.—They are worms without mouth and intestine and are often composed of a long chain of individual animals. *The anterior segment, the head or scolex,* presents a peculiar structure; it is provided with suckers which serve as a means of fixation, and sometimes a wreath of hooklets. *This first segment is to be looked upon as the mother of all the rest, since it produces the whole chain, strobilla,* by budding and fission, so that the oldest segment is farthest removed from the head and the youngest immediately adjacent thereto. This order of arrangement also explains the difference in sexual development of the *individual segments.* The youngest show no sexual glands; the middle segments inclose fully developed sexual organs which in the end segments revert to the originally much smaller uterus, which is here developed into an extensive and often multiramified oviduct.

The individual segments are always hermaphroditic. The opening of the sexual passage is either at the side (tænia) or in the middle line (bothriocephalus) of the segment. Here and there the unsheathed penis can also be seen.

The mature segments *(proglottides)* are capable of independently leaving the intestine, and then manifest quite a strong musculature (tænia saginata), or they escape externally in the feces (tænia solium). *They contain many thousands of ova, within which the embryos are already fully developed.* If the ova enter an appropriate host (cattle, swine, pike) the embryos are set free, penetrate the intestinal wall of the host by means of their six hooklets, and pass with the blood-current to the other organs. They then develop into a vesicle, which often attains considerable size, and multiply by budding. Usually but one bud is produced within the cyst, but not infrequently many hundreds are formed, each one of which presents the organization of a scolex. If such a vesicle (cysticercus, echinococcus) enters the intestine of the original tapeworm host, the vesicle is digested and the scolex again begins to produce a segmented chain by the process of budding.

The cestodes are nourished by the chyme through endosmosis. Their products of metabolism or the toxins pro-

duced by their death and subsequent putrefaction are probably more injurious than the simple abstraction of juices by the worm itself.

Tænia Solium.

This worm (Fig. 61) measures 3 to 3.5 meters in length and up to 8 millimeters in breadth. It is composed of 800 or more segments, of which 80 to 100 are sexually mature. It is distinguished by a *wreath of hooklets* placed in front of the poorly developed suckers. According to Leuckart, these hooklets may occasionally fall off. *Compared with the saginata, the uterus is but slightly branched.* The ova are round and have a thick shell.

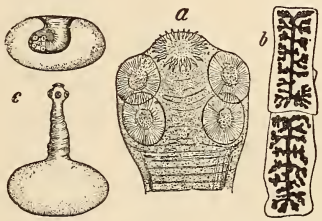


Fig. 61.—Tænia Solium. (After *Leuckart.*)

a, Head. *b*, Proglottides. *c*, Cysticercus cellulosæ (invaginated and protruded).

The proglottides passed in the stools enter the intestinal canal of *swine.* The embryos liberated from the enormous number of ova attack the muscles of the animal and develop into vesicles 8 to 10 millimeters in size, which are designated as *cysticercus cellulosæ,* or *swine* or *pork measle. Man is also subject to invasion by the cysticercus.* If sexually mature proglottides gain admission (by autoinoculation) *per os* to the *human stomach* (antiperistalsis during vomiting is also said to favor this?), the liberated embryos can wander to the various portions of the body. Besides cysticerci of the skin, vesicles leading to serious disturbances are found in the heart, brain, and eyes. In the cysticercus vesicles (*e.g.,* those removed from the skin) the head is always invaginated (Fig. 61, *c*). By gentle or firm pressure and stroking with a brush dipped in water the scolex can usually be made to protrude (Fig. 61, *c*).

Tænia Saginata.

This worm (Fig. 62) is *from 7 to 8 meters long and sometimes possesses from 1200 to 1300 segments from 12 to 14 milli-*

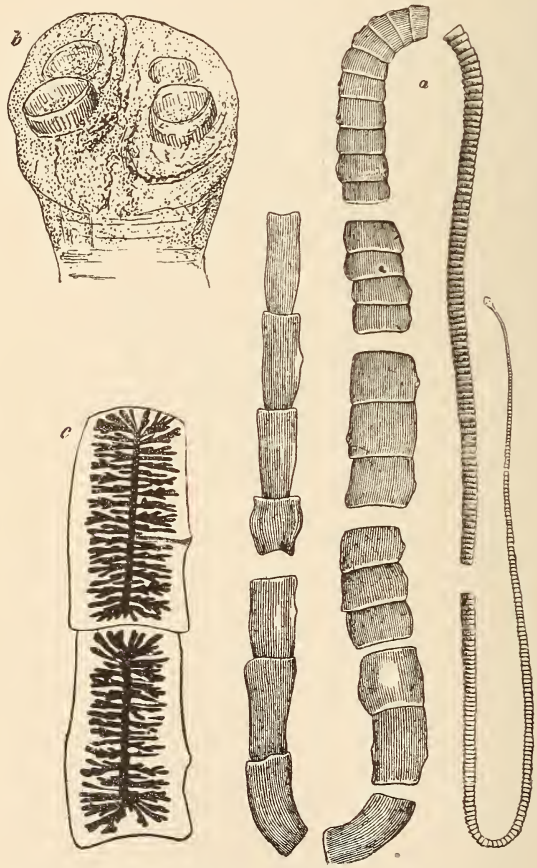


Fig. 62.—Tænia Saginata. (Partly after *Leuckart.*)

a, Natural size of the worm at different sections. *b*, Head (with pigment canaliculi). *c*, Proglottides.

meters broad, of which 150 to 200 are mature. The head presents at its middle a pitlike excavation (no hooklets) and four highly developed muscular suckers. There is occasionally observed upon the head more or less diffuse and intense *pigmentation,* which is

usually attributed to the imbibition of iron salts (from medicaments); whether this view is well founded or not is still undecided. The mature segments which *frequently pass spontaneously* or with the feces, are very muscular; the *uterus presents very numerous branchings.* The elliptic ova, in addition to a strong shell, usually possess a clear (yelk) skin. The excreted segments possess the power of creeping high up on blades of grass, with which they are eaten by cattle, in whom they develop into a

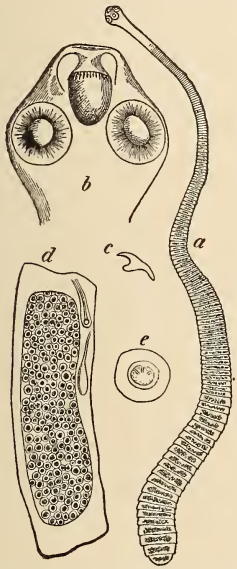


Fig. 63.—Tænia Nana. (After *Leuckart.*)

a, The whole worm (×9). *b*, Head (×50). *c*, Hooklet (×300). *d*, Segment (×50). *e*, Egg (×125).

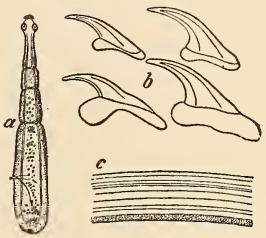


Fig. 64.—Tænia Echinococcus of the Dog. (After *Leuckart.*)

a, Tænia. *b*, Hooklets. *c*, Membrane fragment.

cysticercus resembling externally the cysticercus cellulosæ, but, of course, having the head of tænia saginata. Their development in man has thus far *not* been observed.

Tænia saginata (according to the very reliable observations of Perroncito) grows during the *first* month about 3 centimeters, and in the *second* month when the parasite attains maturity, about 14 centimeters; in other words, each day about 13 proglottides are added.

Tænia Nana.

This parasite (Fig. 63) is the smallest tapeworm thus far observed in man. It is *0.5 millimeter broad* and *at most 15 millimeters in length.* It is composed of from 150 to 170 segments, of which from 20 to 30 are mature. The head presents four rounded suckers and a retractile rostellum armed with a wreath of hooklets.

The worm frequently occurs in large numbers in the intestine and may then give rise to severe nervous disturbances. Lutz observed in little children constant diarrhea accompanied by febrile attacks, which ceased after removal of the worms, which were usually present in large numbers. In the expelled tapeworms the head is frequently overlooked; examination upon a black plate is, therefore, absolutely necessary. This tapeworm was first observed in Egypt and Servia, then quite abundantly in Italy, and *recently also in one instance in Germany, by Leichtenstern, of Cologne.* The mode of development is still undetermined (the cysticercus is probably present in snails, which are here and there eaten raw).

Tænia Echinococcus.[1]

This parasite (Fig. 64) is a tapeworm only 3 or 5 millimeters in length. Many thousands of them often live in the intestine of the *dog.* When tænia *ova* enter the *human* gastro-intestinal canal from the dog (by licking, etc.) the embryos develop in the liver, lungs, and all the remaining organs, often forming an enormous *water-vesicle,* which is surrounded by a *white,* elastic, distinctly *lamellated* wall of variable thickness. Upon the inner surface of this membrane one or a number of small scolices bud forth. The scolices, however, are not always thus directly developed, but are often formed within daughter-cysts. If these be examined under the microscope there will be seen between the four suckers an invaginated wreath of hooklets composed of two series of hooks. Otherwise the vesicle contains a transparent, watery fluid which is distinguished by the *absence of albumin* and the *presence* of sodium chlorid [and succinic acid—Brooks].

[1] See Fig. 64.

For the diagnosis of echinococcic cyst the discharge of whole vesicles is decisive; otherwise attention should be directed to fragments of membrane, hooklets, and the fluid (see also section on "Examination of Aspirated Fluids," page 383).

Bothriocephalus Latus.[1]

This parasite may attain a *length* of from 8 to 9 meters, but, as a rule, it is much shorter. It possesses 3000 and more segments, and is from 10 to 20 millimeters in width and thicker in the middle line than at the margins. The sexual aperture lies in the *median line*. The head is flattened and possesses upon the sides two shallow suction grooves.

The oval eggs (Fig. 56) are 0.05 millimeter long, and 0.035 millimeter broad, and inclosed by one shell only, which is provided with a *lid* or operculum. After they have entered water the ciliated embryos develop and, swimming in the water, invade the *pike* and its musculature, where they grow to scolices, which may attain a size of 10 millimeters.

In the stools of persons affected with bothriocephalus the relatively large ova can readily be seen (see "Examination of Feces"). In addition to numerous perfectly preserved ova, not a few are met with in which the lid has burst open. In others the lid is so firmly adherent that a fissure, produced probably by pressure upon the cover-glass, separates the lid and contiguous eggshell.

Infection of man takes place through the ingestion of insufficiently smoked, boiled, or roasted pike. Aside from the local intestinal disturbances, *severe anemia (q. v.)* is known to occur as the result of bothriocephalic invasion. For this reason, in all cases where doubt exists, a careful search should be made for the ova or, after previous teniafuge treatment, for the characteristic tapeworm segments.

(c) Trematodes.

The trematodes are parenchymatous sucking worms with blind intestine and several suckers. For the practitioner a limited interest is aroused by distoma hepaticum and lanceolatum,

[1] See Fig. 65.

the ova of which are found in the feces. Distoma hæmatobium s. Bilharzia hæmatobia and *distoma pulmonale,* which are very prevalent in tropical countries, especially in Japan, China, India

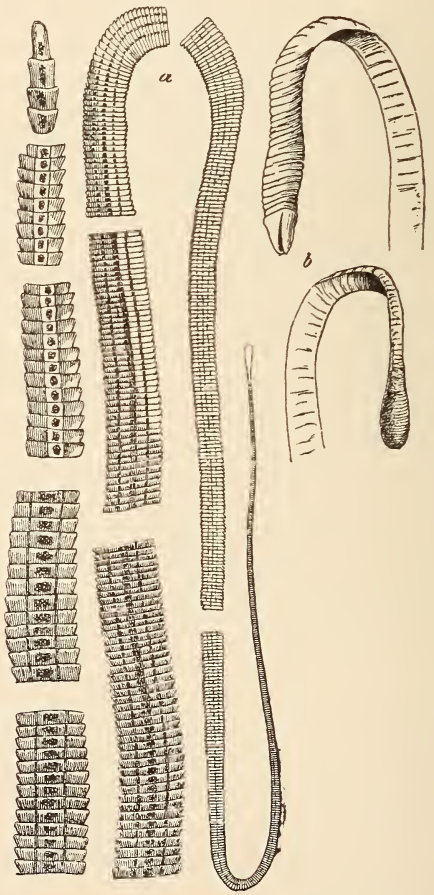


Fig. 65.—Bothriocephalus Latus. (After *Leuckart.*)

a, Worm, in sections; natural size. *b*, Head; lateral and front views.

[and Africa, Brazil, West Indies] are forms which are of greater importance, because of the decided disturbances caused by their presence in the body.

1. Bilharzia Hæmatobia (Fig. 66).—The sexes are separate. The female is from 16 to 20 millimeters long, cylindric, and is carried [for purposes of fecundation] in a deep channel *(canalis gynæcophorus)* located upon the ventral surface of the male. The male worm measures from 12 to 15 millimeters in length. The mature worms inhabit the trunk and

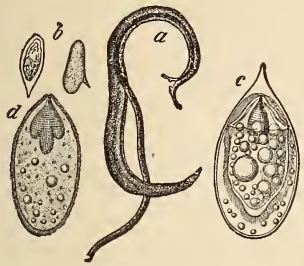


Fig. 66.—Bilharzia Hæmatobia. (After *Leuckart.*)

a, Male and female in copulation (× 10). *b*, Ova with polar and lateral spicula (× 12). *c*, Ovum containing embryo (× 40). *d*, Free embryo with cilia (× 50).

Fig. 67.—Egg of Distomum Pulmonale, Lid Sprung. Sputum Preparation.

branches of the portal vein and the venous plexuses of the urinary bladder and rectum. On the other hand, the ova, besides being found in the localities mentioned, also occur in the bladder-wall, free in the bladder, and—what *is especially important from a clinical standpoint*—in the *urine, which generally presents the signs of cystitis with hematuria.* As the venous vessels are often densely filled with these parasites, conges-

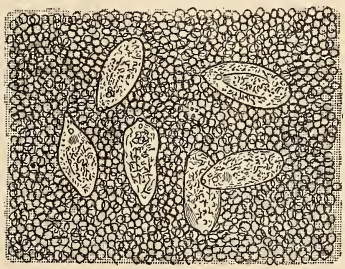


Fig. 68.—Eggs of Distoma from Urinary Sediment. (After *Purdy.*)

tion and stasis, with escape of blood and ova, occur. *The latter measure 0.05 millimeter in breadth, 0.12 millimeter in length, and carry at one end a long, sharp spicule* (ova with a *lateral* spine are often seen in the bladder-wall. The eggshell is moderately thick and *without a lid.* Within the free ovum the embryo is visible and often manifests active

movements. *It escapes from the shell only when the ovum enters water*, and there it bursts the shell longitudinally. It has a wedge-shaped form, a cephalic papilla, and is covered with numerous cilia. The embryos appear to be nonmotile in the *urine*, in which they die after twenty-four hours. Transmission very probably occurs through the agency of drinking-water, in which, as has already been said, the embryos escape from the ova within a few minutes. Whether the embryos first require an intermediate host or not is still undetermined.

The parasite was discovered by Bilharz, in 1851, in Cairo, Egypt. Our knowledge of its biologic characteristics, etc., has been increased by the investigations of Chatin, Sonsino, and especially by Leuckart.

2. Distoma Pulmonale (Fig. 67).—The 8- to 11-millimeter long worm is chiefly located in the upper air-passages, sometimes within small cavities in the lungs which have been produced by it. It is cylindric in form; the anterior end is decidedly rounded, the posterior less so; and possesses an oral and an abdominal suction disc.

The ova are elliptic and possess a *brownish-yellow* shell from $^1/_2$ to 1 μ thick, within which the embryo is not fully developed, but segmentation has already occurred. On pressure upon the cover-glass the shell bursts and the contents escape and can readily be seen, with a hand magnifying glass, as light-brown points averaging 0.04 millimeter in breadth and 0.06 millimeter in length (Scheube). In an old preparation placed at the author's disposal by Scheube several were found to be only 0.016 millimeter broad and 0.026 millimeter long.

Patients affected with this worm suffer most frequently from recurrent *hemoptysis*. The sputum, which is usually raised only in the early morning by hawking, is sometimes tough and mucoid and streaked with blood, sometimes only bloody. *It always contains numerous* (in one preparation one hundred and over) ova like those described above, and, as a rule, quantities of Charcot-Leyden crystals.

II. EXAMINATION OF THE BLOOD.

(A) THE BLOOD IN HEALTH.

Healthy arterial blood shows the bright color of oxyhemoglobin, due to oxidation of the hemoglobin. The more oxygen it loses, the darker it becomes. Nonoxygenated blood is dichroic; it appears dark red by transmitted light and green by reflected light. Oxyhemoglobin is formed by merely shaking a solution of hemoglobin in the presence of air, but it readily parts with its oxygen, especially in the presence of yellow ammonium sulphid and salts of copper.

The Reaction of the Blood during Life is Always Alkaline.—If a strip of red litmus-paper thoroughly moistened with concentrated solution of sodium chlorid is drawn several times through blood and then the adherent blood quickly washed off with the salt solution, a distinct alkaline reaction will, as a rule, be seen. Delicate alabaster or clay platelets, saturated with litmus tincture, upon which a drop of blood is allowed to act for a *moment* before it is washed off with water, are used for the same purpose. Landois's method is quite well adapted for the estimation of the *degree* of alkalinity.

[**Dare's Hemo-alkalimeter.**—Quite recently Dr. Arthur Dare has perfected an instrument for determining the degree of alkalinity of the blood. The description of the apparatus and its use as given by the inventor is as follows:—

"The first part (Fig. 68*a*, *B*) consists of a stopper through which passes an automatic capillary blood-pipette, containing 20 cubic millimeters by volume or 15 milligrams by weight (of normal blood), the exposed end of which is ground to a tapering point. The caliber of this little tube remains the same throughout. This stopper and the contained blood-pipette are fitted into a clear glass test-tube (Fig. 68*a*, *A*) containing 3 cubic centimeters, the upper end of which is expanded into a bulb containing on its side a minute opening (Fig. 68*a*, *C*) for the purpose of admitting air. The top of the bulb is placed close to the stopper, allowing the capillary tube to pass to the center of the bulb. The test-tube is graduated in cubic centimeters, and the equivalents in milligrams of NaOH to 100 cubic centimeters of blood are also represented.

"The second part of the instrument, *the reagent pipette*, consists of an appliance made up of a glass tube terminating with a rubber bulb and having at its other extremity a piece of rubber tubing which fits over the sharpened end of the capillary blood-pipette previously described. In

conjunction with this instrument a spectroscope should be used, the Queen pocket spectroscope answering all purposes.

"The test solution to be employed is made up as follows:—

Acidi tartarici (Merck's reagent)	gr. j	0.065	gm.
Alcoholis	ʒvj	24	c. cms.
Aquæ destillatæ	q. s. ʒviss	200	c. cms.

0.375 to a liter $^1/_{200}$ of normal.

The alcohol is added to prevent the formation of fungous growth, but not in sufficient quantity to precipitate the albumin of the blood in any morbid condition.

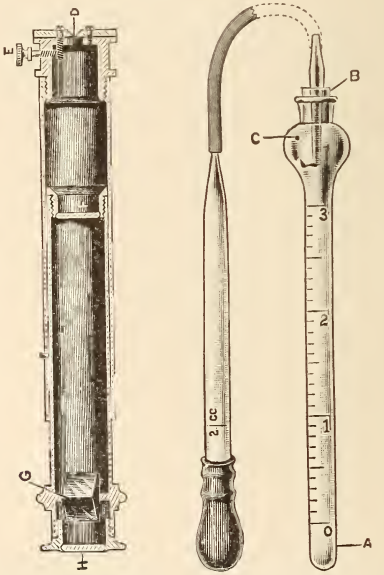


Fig. 68*a*.—Hemo-alkalimeter. (About one-half actual size.)

A, Alkalimeter tube. *B*, Automatic blood-pipette. *C*, Opening for the admission of air. *D*, Cleft of spectroscope, into which the alkalimeter tube is placed at the point *A*. *E*, Cleft adjustment. *F*, Diaphragm to intercept reflected light. *G*, Nicol prism. *H*, Eyepiece.

"Method of Employing the Instrument.—A drop of blood is obtained from the finger-tip or lobe of the ear in the usual manner. The test-tube is held horizontally, and its contained blood-pipette fills automatically by capillary attraction when the sharpened end is touched to the blood-drop as it emerges from the wound. With an ordinary minim pipette, with a piece of rubber tubing over the free extremity, wash this column of blood into the test-tube with distilled water up to the point 0 (zero), which is the first division from the bottom, holding the test-tube

vertically. Close the opening in the bulb of the test-tube with the thumb and invert the tube several times, to cause a thorough mixing of the blood and water. The *reagent pipette* is filled with the acid reagent, and the rubber tubing is fitted over the sharpened end of the blood-pipette; by compressing the rubber bulb the acid solution is forced through the pipette into the test-tube, the aperture in the glass bulb being closed before the pressure is relaxed, to prevent the mixture of the acid solution and the blood. Having done this, the test-tube is inverted several times while still being attached to the reagent pipette, care being exercised to keep the reagent pipette in a vertical position, to avoid a gravitation of the acid solution into the rubber bulb of the reagent pipette, thus preventing a chemic action upon the rubber nipple with the formation of a flocculent precipitate. The interval (Fig. 68*a*, *A*) between the closed end of the tube and the first marking *(0)* should be placed in the cleft of the spectroscope (Fig. 68*a*, *D*) and observation be taken as to the existence of bands of oxyhemoglobin. Should these bands be present the careful addition of acid solution is necessary. As the bands become fainter and fainter we know that we are approaching the point of neutralization, and it becomes necessary to add the acid, 1 drop at a time; then invert the test-tube and examine with the spectroscope as before. After performing several tests with this instrument it will not be necessary to apply the spectroscope so frequently to determine the point of neutralization, as the eye quickly learns to detect this characteristic change by the color of the blood-mixture. When the bands of oxyhemoglobin disappear the observation is at an end. It is then only necessary to read the result from the scale on the test-tube, which is graduated in cubic centimeters and the equivalents expressed in milligrams of sodium hydrate to 100 cubic centimeters of blood.

"Scale of Equivalents Computed from a Basis of 15 Milligrams of Blood to 2 Cubic Centimeters of Acid Solution ($^1/_{200}$ of the Normal).

C. cms. of Reagent.	Mgs. of NaOH to 100 c. cms. of Blood.
2.6	345
2.4	319
2.2	292
2.0	266
1.8	239
1.6	212
1.4	176
1.2	159
1.0	133
0.80	96
0.60	79
0.40	53
0.20	26.6

"As a uniform light is necessary for physiologic experiments, artificial illumination should be selected as being always available; an open gaslight was employed in our experiments entirely, and the relation of the spectroscope to the source of illumination kept always constant."—Brooks.]

The **specific gravity** varies within narrow limits. The average is 1.055.

It is most accurately estimated by Schmaltz's (pyknometric) method, in which only 0.1 gram, about 2 drops, of blood is employed. A fine capillary glass tube about 12 centimeters long, 1.5 millimeters thick, and drawn out at the ends to 0.75 millimeter is accurately weighed upon a chemic scales, then filled with distilled water, and again weighed. The capillary tube is then cleansed, filled with blood, and the weight again determined. The number obtained after subtracting the weight of the capillary tube is divided by the accurately determined weight of the equal amount of distilled water. The quotient shows the specific gravity of the blood.

While this method requires very delicate scales, such as are found only in well-equipped laboratories, the *method of Hammerschlag can be practiced by any physician.* It is based upon the law that a body which floats stationary within a fluid possesses the same specific gravity as the fluid. For this purpose a mixture of chloroform (spec. grav., 1.485) and benzol (spec. grav., 0.889) is employed, with which the added drop of blood does not mix.

Technique for the Method of Hammerschlag.—A glass cylinder about 10 centimeters high is half filled with a mixture of chloroform and benzol having a specific gravity of 1.050 to 1.060. Into this fluid a drop of fresh blood drawn with a lancet is allowed to fall without touching the wall of the cylinder. The drop will either sink to the bottom as a red bead or rise to the top. In the former instance, because the surrounding fluid is lighter than the blood, chloroform is added *drop by drop;* in the latter case benzol; while by careful agitation of the glass it is sought to make the drop float stationary in the body of the liquid. When this is attained the specific gravity of the blood and the mixture is equal and can be determined with an areometer in the same cylinder or, after filtration, into a taller cylinder. The chloroform-benzol mixture is unaffected by this procedure and can be used repeatedly.

Hammerschlag advises the observance of certain precautions which the author fully indorses. Not too large a drop should be taken, for such will much more readily break up into a num-

ber of smaller ones. Care should likewise be observed during agitation of the cylinder to secure thorough admixture of the benzol and chloroform and also to avoid splitting of the drop of blood. Finally, in every case in which the blood floats upon the surface from the beginning, it is advisable to cause the drop to sink by adding an excess of benzol and then to make it float stationary in the fluid by gradual admixture of chloroform.

By frequent repetition of the process quite a degree of dexterity can soon be acquired; so that the method is strongly recommended to every physician accustomed to make careful examinations of the blood.

[The following tables give the percentage of hemoglobin as indicated by the specific gravity:—

I. (HAMMERSCHLAG.)

SPEC. GRAV.	HEMOGLOBIN.
1.033—1.035 =	25—30 per cent.
1 035—1.038 =	30—35 "
1.038—1.040 =	35—40 "
1.040—1.045 =	40—45 "
1 045—1.048 =	45—55 "
1.048—1.050 =	55—65 "
1.050—1.053 =	65—70 "
1.053—1.055 =	70—75 "
1.055—1.057 =	75—85 "
1.057—1.060 =	85—95 "

II. (SCHMALTZ AND PEIPER.)

SPEC. GRAV.	HEMOGLOBIN.
1.030 =	20 per cent. + —
1.035 =	30 "
1 038 =	35 "
1.041 =	40 "
1.0425 =	45 "
1.0455 =	50 "
1.048 =	55 "
1 049 =	60 "
1.051 =	65 "
1.052 =	70 "
1.0535 =	75 "
1.056 =	80 "
1.0575 =	90 "
1.059 =	100 "

—BROOKS.]

The **total amount of blood** in the human body amounts to one-thirteenth of the body-weight.

Composition.—The blood consists of the plasma, holding the fibrin in solution, and the cellular elements: the red and white blood-corpuscles and blood-plates.

The **red blood-corpuscles, erythrocytes** [ἐρυθρός, red; κύτος, cell], consist of the stroma, which is readily soluble in ether and chloroform, and the **hemoglobin** upon which the color of the blood depends. The latter is an albumin body closely related to globulin. It is combined with an iron-containing coloring matter which can readily be extracted by acids and strong alkalies as *hematin*. While this substance is, as a rule, obtained only as an amorphous, brown powder, its chlorin derivative, *hemin*, can be secured in characteristic crystalline form. Hemoglobin

can be separated from the stroma of the blood-cells by high temperature, electricity, decanting with carbonic acid water, intravascular infusion of serum of another species of animal, and other procedures. The blood assumes a "laky" tint. *If this occurs in the living body, great danger is threatened by the liberated stroma* (Alexander Schmidt).

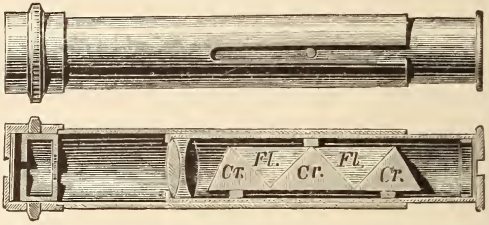


Fig. 69.—Browning Hand Spectroscope. *(Zeiss.)*

Healthy blood shows a decided spectroscopic character, which can readily and quickly be determined with the hand spectroscope (Zeiss) (Fig. 69). A few drops of blood are mixed with water in a test-tube, and the latter is held in front of the slit of the spectroscope, which is held toward the light. At once absorption-bands appear (between the lines *D* and *E*) in the yellow and green of the spectrum (Fig. 70, *a*).

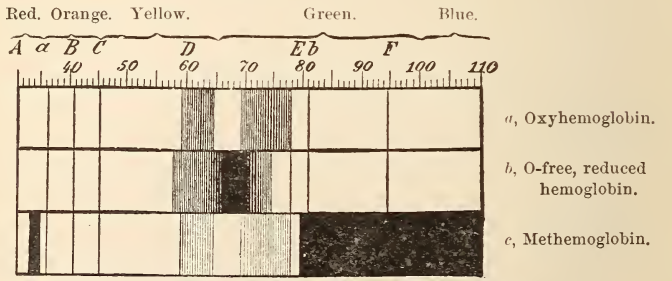

Red. Orange. Yellow. Green. Blue.
A a B C D E b F
40 50 60 70 80 90 100 110
a, Oxyhemoglobin.
b, O-free, reduced hemoglobin.
c, Methemoglobin.

Fig. 70.—Spectrum with the Zeiss Hand Spectroscope.

If a few drops of a solution of yellow ammonium sulphid or cupric sulphate are added, the bands disappear and in their place is seen a broad band peculiar to deoxidized, *reduced hemoglobin* (Fig. 70, *b*). By shaking in the presence of air or stirring the mixture with a glass rod, the absorption-bands of oxyhemoglobin reappear.

Microscopic examination of the blood is conducted (1) with freshly drawn blood or such as has been preserved in a moist chamber; (2) with dried-blood preparations.

Microscopic Examination of Fresh Preparations. — Carefully cleanse the finger-tip or, as the author generally prefers, because of less liability to infection, the lobe of the ear, and draw a small drop of blood with a lancet [needle or other convenient instrument]. With a pair of forceps grasp a cover-glass, touch it to the drop of blood, and then place it as soon as possible upon a glass slide. It is advisable so to select the drop that the whole space between the cover-glass and slide is occupied by a uniform, delicate layer of blood; pressure upon the cover-glass should be avoided.

With a magnification of about 250 or 350 the red blood-corpuscles can be seen isolated and in rouleaux, and here and there occasional colorless blood-cells [leucocytes: λευκός, white; κύτος, cell]; finally, small, pale, round or elliptic shaped bodies which were first described by Bizzozero as blood-plates.

The red blood-corpuscles[1] **(erythrocytes)** are biconcave disks with rounded edges. They appear as circular plates when viewed from the flat surface, as biscuit-shaped bodies when seen from the edge. On careful focusing the flat surface of the cells shows a central depression as a dim shadow, becoming darker toward the periphery; otherwise the center of the cell is bright and the margin darker. By arrangement of a number of blood-cells upon each other, distinct columnar or rouleaux formation is produced [see Fig. 71]. The individual cells are nonnucleated,

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[1] Virchow's statement—"*Die Geschichte der röthen Blutkörper ist immer noch mit einem geheimnissvollen Dunkel umgeben*" [the history of the red blood-corpuscles is still enshrouded in mysterious darkness]—is, unfortunately, still true to-day. The view that the red and colorless blood-cells are from the beginning two separate cell-groups is the most worthy of consideration.

According to the older view, which has recently been defended by H. Müller, the red and colorless blood-cells originate from a single variety of colorless cells, which develop into leucocytes as well as erythrocytes—in the latter instance by the reception of hemoglobin. On the other hand, Denys and Löwit assume the existence of two different kinds of colorless elementary cells (leuco- and erythro- *blasts*) and differ in their views only as to the locality in which the transformation of the colorless into colored corpuscles occurs. According to Denys, who attributes karyomitosis (indirect nuclear division) to both types, the transformation takes place only in the bone-marrow; according to Löwit, only in the circulating blood. Hayem, however, considers the hematoblasts (blood-plates) as the sole antecedents of the red blood-corpuscles, while Neumann and Bizzozero reject all these views and assert that the red blood-corpuscles originate entirely through mitosis of young, nucleated red cells within the bone-marrow.

homogeneous, and yellowish green in color. According to the thickness of the preparation there will sooner or later appear near the margin of small air-bubbles or the cover-glass red blood-corpuscles with serrated margins [crenation] or in the form of thorn-apples; this is a sign pointing to the occurrence of evaporation. Such bodies are also very rapidly produced on addition of sodium sulphate, while addition of water causes a spheric swelling of the corpuscles, with disappearance of the central concavity.

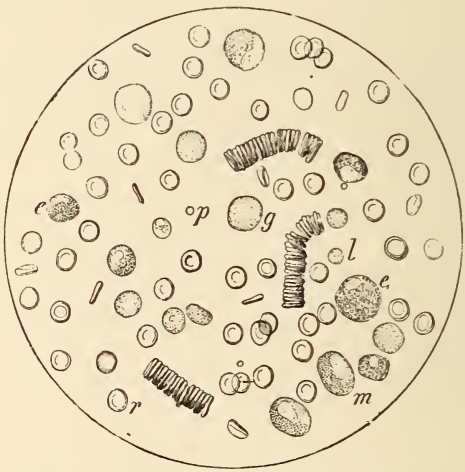


Fig. 71.—Leukemia with Large Spleen Tumor. × 350.

r, Red blood-corpuscles. *e*, Normal; e_1, pathologic eosinophile cells. *g*, Finely granular leucocytes. *m*, Marrow-cells. *l*, Lymphocytes. *p*, Blood-plates.

As a rule, the red blood-corpuscles are quite uniform in *size* and form. According to numerous researches by Gram, Laache, Gräber, and others, the average size is 7.8 μ, and is the same in men and women. Gram found the diameter most frequently to be 7.9 μ, and rarely 9.3 μ. The smallest diameter may be assumed to be 6.5 μ.

The white, or colorless, blood-corpuscles (leucocytes) always occur isolated in the field of the microscope. Even without the addition of reagents fine granulations and irregularities of the surface can be recognized in them. The nuclear figures, which are often visible in fresh preparations, are plainly brought to

view on addition of dilute acetic acid. When examined upon the warm stage the leucocytes often manifest active independent motility [ameboid movements].

According to the size of the cells, which may be between 3 and 15 μ, and the form and number of their nuclei, four kinds of leucocytes are generally distinguished:—

1. Small, mononuclear, round cells with comparatively large round nucleus and small, noncontractile peripheral mass of protoplasm. The cells are smaller than the red blood-corpuscles. They are designated as **small lymphocytes.**

2. Large mononuclear cells with pale cell-body, at least the size of a red blood-corpuscle or somewhat larger. The nucleus is usually ovoid or crescent formed, and sometimes shows beginning lobulation **(large lymphocytes).** (See Plate IX, Fig. 16.)

3. Leucocytes with a somewhat more refractive, *finely granular,* contractile protoplasm and multiformed nuclei **(polymorphonuclear leucocytes).** If the nucleus is so divided that the individual parts are no longer united, then the cells are designated as **polynuclear leucocytes.** These two forms constitute the great majority of the colorless blood-corpuscles, and they may be designated as leucocytes in a strict sense.

4. A small number of the forms described under No. 3 are distinguished by decidedly intense, glistening granulation of the cell-body **(coarsely granular leucocytes**—Max Schultze; these are the cells which Ehrlich designates as **eosinophiles**; see Fig. 71, *e*).

It may here be remarked that the analytic staining method of Ehrlich has resulted in a division of the above-mentioned polymorphonuclear and polynuclear leucocytes into three groups, as follows:—

1. Leucocytes with neutrophilic granules.
2. Leucocytes with eosinophilic granules.
3. Leucocytes with basophilic granules.

(Compare pages 160 *et seq.*)

The **blood-plates** discovered by Bizzozero are very unstable bodies, with a tendency to rapid disintegration. Since they collect at the margin of the wound very rapidly after puncture, the first drop of blood that exudes must always be employed for

examination. Even then they are readily overlooked, because they rapidly disintegrate in blood preparations made in the ordinary way.

In order to secure them in an unaltered state, it is advisable to collect the first drop of blood directly in a preservative solution without exposure to air. For this purpose the following are recommended: 1. A 1-per-cent. watery solution of osmic acid. 2. A mixture composed of 1 part of this and 2 parts of physiologic salt solution. 3. A 14-per-cent. solution of magnesium sulphate. 4. A solution of 1 part of methyl-violet in 5000 parts of physiologic salt solution. With the latter solution the bodies appear well stained. [This may also be done by placing a drop of the preservative solution upon the finger-tip and puncturing through the drop. The exuding blood mixes with the solution and the mixture can then be examined under a cover-glass on a slide.—Brooks.]

In unstained preparations the plates appear as pale-red, slightly glistening, circular or ovoid disks, 1.5 to 3.5 μ in size. They lie chiefly in groups of from four to six.

At the present time diagnostic significance cannot be attributed to them, for the reason that the views as to whether the plates are integrant constituents of normal blood (Bizzozero, Hayem, Afanassiew, and others) or disintegration products of colorless blood-corpuscles (Löwit, Weigert, and others) are too much at variance. There is, however, no question but that the view of Hayem, who looks upon the plates as the antecedents of the red blood-corpuscles and therefore designates them hematoblasts, must be wholly rejected.

Not infrequently fine, pale bodies much smaller than the blood-plates are met with in specimens of blood prepared *without* preservatives. These are generally designated as **elementary granules.** They are proteid bodies, and are not rarely arranged in small groups. They are quite justly considered as disintegration products of the leucocytes and blood-plates for the reason that they do not occur in specimens prepared under the precautions above mentioned.

The preparation and staining of *dried blood specimens* will be considered when discussing the examination of diseased blood.

Counting of the cells occurring in normal blood is of the greatest importance. As a rule, an estimate of the number of corpuscles in one cubic millimeter of blood is made according to the method of Vierordt,

Welcker, and others. As the undiluted blood is wholly unadapted for this purpose, because of the myriads of blood-corpuscles and the tendency of the latter to form rouleaux and undergo rapid change of shape, each count must be made with a more or less greatly diluted sample of blood. The following solutions are adopted for diluting:—

1. A 3-per-cent. solution of sodium chlorid.
2. A 15- to 20-per-cent. solution of magnesium sulphate.
3. A 5-per-cent. solution of sodium sulphate.
4. **Hayem's solution:**—

Hydrarg. bichlor.	0.5
Sodii sulphat.	5.0
Sodii chlorat.	1.0
Aq. destill.	200.0

5. **Pacini's fluid:**—

Hydrarg. bichlor.	2.0
Sodii chlorat.	4.0
Glycerini	26.0
Aq. destill.	226.0

Each solution is applicable. Hayem's solution deserves to be especially recommended, because it preserves the form and color of the blood-corpuscles with scarcely any alteration in their size.

Counting is most conveniently done with the Thoma-Zeiss counting apparatus, which may be regarded as a combination of the apparatus of Malassez, Hayem, and Gowers. It consists of a mixer (Fig. 72, *M*, *S*), a glass slide with counting chamber *(O)*, and a plane cover-glass *(D)*.

Counting Red Blood-corpuscles.—By means of the rubber tube attached to the mixer, upon which the numbers *0.5*, *1*, and *101* are engraved, suck up the blood to the mark *0.5* or *1*, then hastily wipe off the tip of the pipette and as quickly as possible draw up the diluting solution to the mark *101*. In sucking the blood into the pipette great care should be observed *not* to draw in air along with the blood or to allow the diluting solution to go beyond the mark *101*. [The Grawitz pipette overcomes the latter source of error.—Brooks.] When the bulb *(E)* is filled up to this mark, the fluid must at once be mixed as thoroughly as possible, which is accomplished by shaking the pipette and by the movements of the glass bead contained in the mixing chamber. During shaking the lower opening of the pipette should be closed with the finger and the rubber tube compressed just above the mark *101*. According to whether the blood has been drawn up to the mark *0.5* or *1*, the dilution will be 1 to 200 or 1 to 100, respectively, because the capacity of the bulb between the marks *1* and *101* is exactly 100 times greater than the contents of the capillary tube from the tip *S* to the mark *1*.

Before a drop of the blood-mixture is placed in the counting chamber, the fluid in the capillary tube of the mixer must first be blown out, because it consists only of unmixed diluting solution. A drop of the

blood-mixture is then placed in the counting chamber and carefully covered with a cover-glass, care being taken that *no air* is admitted and that the drop just fills the space between the cover-glass and disc [none running over into the channel surrounding the disk—Brooks]. After waiting a few minutes for the blood-corpuscles to settle, the counting is begun with from 120 to 200 magnification. [In counting the corpuscles, a Leitz 6, Bausch and Lomb $^1/_6$, or Spencer 4 millimeter lens should be used, as these take in just one field of sixteen small squares, including the lateral division lines.—Brooks.]

The counting chamber *(b)* shows the following arrangement: Upon a strong, smooth ground slide *(O)* is cemented a square glass plate *(W)* with a central circular opening. Upon the bottom of the cavity thus formed is set a delicate glass disk *(B)*, which is exactly 0.1 millimeter less in thickness than the surrounding plate *(W)*. The upper surface of this

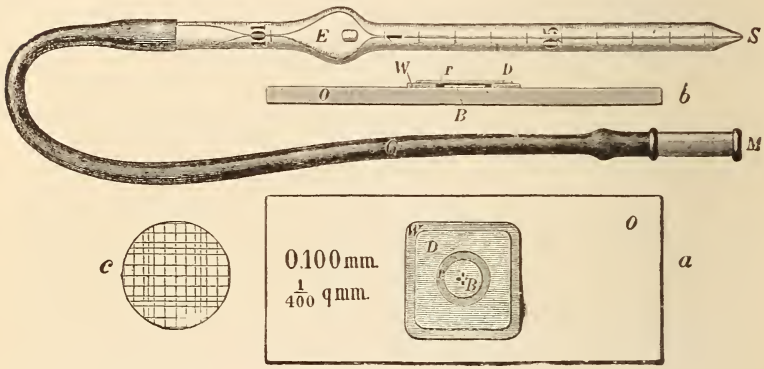


Fig. 72.—Thoma-Zeiss Blood-counting Apparatus.

disk presents a microscopic scale as seen in Fig. 72, *c*. This is composed of sixteen large squares, each of which incloses sixteen smaller squares. With the exception of those situated at the external margins of the ruled lines, each large square is separated from the others by a double row of rectangles to facilitate counting. [The counting chamber recently introduced by Türck is much more convenient, since it has separate divisions for counting the leucocytes synchronously with the red cells (see Fig. 74).—Brooks.]

In counting it is best to take each of the four superimposed rows of small squares in order and count all the corpuscles lying within and upon the upper and left-hand line of each small square, and, finally, add the cells lying upon the lower and right-hand margin of each large square. In order to avoid repetition, it is best to begin with the upper left-hand squares and count a sufficient number of individual squares to give a total count of at least 1100 red cells.

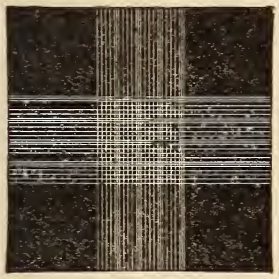
As the depth of the counting chamber is $^1/_{10}$ millimeter and the area of each square $^1/_{400}$ quadrimillimeter, the cubic content of each amounts to $^1/_{4000}$ cubic millimeter. Therefore, in order to calculate the number of red blood-corpuscles in 1 cubic millimeter, the total number of red blood-corpuscles counted is divided by the number of small squares counted and the quotient multiplied by 4000 and then, according to the dilution used, by 100 or 200.

Example: Assuming that 1580 red cells were counted in 128 squares with a blood dilution of 1 to 100, the following would be secured:—

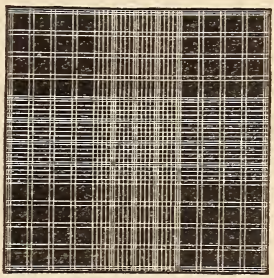
$$\frac{1580}{128} = 12.3 \times 4000 \times 100 = 4{,}920{,}000$$

Therefore, the blood examined would contain 4,920,000 red blood-corpuscles in 1 cubic millimeter.

According to the figures obtained by the Thoma-Zeiss method the average for a man is 5,000,000, and for a woman 4,000,000 to 4,500,000 red blood-corpuscles per cubic millimeter. When skillfully and carefully



[Fig. 73.—Thoma-Zeiss Counting Chamber.]



[Fig. 74.—Türck Counting Chamber.]

conducted, the limits of error vary in this method at most between 1 and 2 per cent. Considerable increase in the number of red blood-corpuscles is, on the whole, rarely observed; however, from 6,000,000 to 7,000,000 red corpuscles per cubic millimeter have been observed in perfectly healthy individuals (plethora polycythemica).

An exact count is possible only when the counting chamber and especially the mixing pipette is scrupulously clean. This can be accomplished only by blowing out immediately after use whatever may still remain in the tube and then washing out successively with the diluting solution, water, alcohol, and ether, these fluids being sucked up into the pipette and then blown out. Finally, the mixer is to be thoroughly dried by forcing air in and out of the empty apparatus.

NOTE.—In the author's opinion, the method recommended by Hedin for the estimation of the number of blood-corpuscles with *hematocrit* offers no advantages; in all diseases in which great variations in the size of the blood-cells exist it is wholly inapplicable. [I fully indorse this view.—BROOKS.]

In counting the colorless blood-corpuscles the same counting chamber is used, but with another mixing pipette. Upon the latter are marked the numbers *0.5*, *1*, and above the bulb *11*. Rieder has had constructed for him by Zeiss a shorter pipette adapted for twenty-one space divisions. For diluting, $^1/_3$- to $^1/_2$-per-cent. acetic acid is used, whereby the red blood-corpuscles are dissolved. A little gentian-violet solution may be added in order to stain the leucocytes and thus facilitate counting. As a rule, the blood is diluted in the proportion of 1 to 10 or 1 to 20; when there is a great increase of leucocytes, as in leukemia, a dilution of 1-25 to 1-50 is advisable. Otherwise the count is made in the same manner as with red corpuscles, except that in the final reckoning the operator must multiply with 10, 20, or 50, according to the dilution employed.

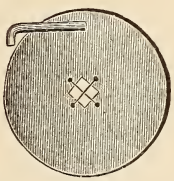
The number thus obtained for the absolute quantity of leucocytes in 1 cubic millimeter of blood varies between 6000 and 10,000; the average count for an adult is 8000; for children, 9500.

The sources of error are greater than in the estimation of the number of erythrocytes. The size of the capillary tube is of itself the source of frequent error. Nevertheless, after much practice and the observance of great care comparatively exact results may be obtained.

The leucocytes may also be counted in an indirect way in the following manner: With the aid of the Zeiss micrometer very carefully count the number of red and white blood-cells in a stained dry preparation. Determine the *ratio* of both kinds of cells and then calculate the total number of *red* blood-corpuscles in 1 cubic centimeter by the Thoma-Zeiss method. When both amounts are ascertained it is easy to compute the number of leucocytes in 1 cubic millimeter. All that is necessary is to divide the number of erythrocytes found in 1 cubic millimeter (see Fig. 72, *S*) by the denominator of the fraction obtained from the ratio of white to red corpuscles:—

If $s = 5{,}000{,}000$ and the ratio of white to red cells is $\frac{1}{700}$ then the number of leucocytes per cubic millimeter will be $\frac{5{,}000{,}000}{700} = 7142$.

[Einhorn-Laporte Blood-counting Diaphragm.—The blood-smears are made in the usual manner (see page 150). Without fixation, stain for from two to three minutes in Jenner's stain. After selecting with a low-power lens (Leitz No. 3, Spencer 16 mm., Zeiss AA) a field in which all the corpuscles are thinly and evenly spread, use the counting diaphragm with a Zeiss DD, ocular 2, or Leitz No. 6, ocular I, or Spencer 4 mm., ocular 4x (see table). Count all the leucocytes in the entire *field,* and all the red corpuscles in the entire *square aperture* or one of its quadrants without moving the specimen. Then move the slide for the width of one field and again count the white cells in the entire field and the erythrocytes in the square. This is to be repeated until about 6 square millimeters (26 fields, Zeiss; 37 fields, Leitz; 46 fields, Spencer) have been counted. To find the **number of leucocytes in 1 cubic millimeter** divide the total num-



[Fig. 75.—Einhorn's Blood-corpuscle Counter.]

ber counted by the number of square millimeters counted and multiply by the average factor, 400. For example: if 416 leucocytes were counted in 6 square millimeters the following would be the procedure:—

$$416 \div 6 = 69.3 \times 400 = 27{,}720.$$

The **number of red cells** is ascertained in the same manner, except that the resulting quotient is multiplied by the figures indicating the number of times the field of the ocular is larger than that of the field of the counting diaphragm. With Zeiss DD lens and ocular 2, this is 16 (Leitz 6, ocular I, 14; Spencer 4 mm., ocular 4x, 10). For example: If 2336 erythrocytes were counted in 6 square millimeters the equation would be as follows:—

$$2336 \times 16 \div 6 = 6229 \times 500 = 3{,}114{,}650$$

TABLE GIVING THE AREA OF FIELD OF VARIOUS LENS COMBINATIONS MOST COMMONLY USED.[1]

ZEISS (Tube-length, 160 mm.).			LEITZ (Tube-length, 170 mm.).			SPENCER (Tube-length, 160 mm.).		
Objective.	Entire Field of Eyepiece 2.	Field Embraced by Square Opening in Blood-counting Diaphragm.	Objective.	Entire Field of Eyepiece 1.	Field Embraced by Square Opening in Blood-counting Diaphragm.	Objective.	Entire Field of Eyepiece 4x.	Field Embraced by Square Opening in Blood-counting Diaphragm.
	sq. mm.	sq. mm.	.	sq. mm.	sq. mm.	mm.	sq. mm.	sq. mm.
DD	0.23	0.0144	5	0.32	0.023	4	0.13	0.013
E	0.09	0.0056	6	0.16	0.011	2	0.035	0.0035
$\frac{1}{12}$ oil	0.05	0.0031	7	0.096	0.0067			
			$\frac{1}{12}$ oil	0.031	0.0022			

—BROOKS.]

There is no *exact* method for *counting the blood-plates*. Enumeration with the Thoma-Zeiss counting chamber gives uncertain results. If, however, it is desirable to undertake the task, it is absolutely necessary to employ one of the above-mentioned preservative fluids. Hayem places the normal number at 240,000 per cubic millimeter, while, according to others, the number varies between 200,000 and 350,000. Accordingly, it may be assumed that they are about thirty-five times more numerous per cubic millimeter than are the leucocytes.

In the **estimation of the (relative) hemoglobin content** Fleischl's hemoglobinometer is adapted for clinical purposes.

The apparatus (Fig. 76) consists of a strong foot supporting a table *(t)* with a central circular opening, through which the light from a gas-flame or candle is reflected from the mirror *(s)*. On the under surface of the table is a metal frame *(r)* which can be moved back and forth in a groove by means of a thumb-screw. In that side of the frame next to the observer is fastened a wedge-shaped strip of glass *(g)* colored with Cassius's "gold-purple."[2] In the circular opening in the table can be adjusted a cylindric cup *(b)* closed at the bottom with glass and separated into two equal chambers by a metal partition. The cup is so placed that the partition stands parallel with the front edge of the table, and the posterior half of the chamber—*i.e.*, that portion next the observer—is exactly over the colored glass wedge. That side of the frame next the colored glass wedge is marked with a scale from 0 to 120, the figures

[1] See Medical News, April 19, 1902, page 741.

[2] [Mixed oxids of gold and tin.]

of which can be read through a small aperture *(a)* in the table when the frame is moved back and forth.

In making an examination both halves of the chamber are filled with water. In the front half immediately over the mirror some blood is dissolved, the amount of which is accurately measured with the accompanying small pipettes. According to Schmaltz, the latter hold about 0.0054 centimeter of distilled water. The color of the glass wedge when set at the scale number 100 corresponds to the color shown by normal blood when diluted in the front chamber as above described. The numbers at the left of 100 indicate what percentage of the normal amount of hemoglobin (which averages in men, 13.7; in women, 12.6 grams in 100 cubic centimeters) is contained in the blood examined.

According to the researches of Dehio, this method of estimation is attended by many errors, which increase with the decline in the amount

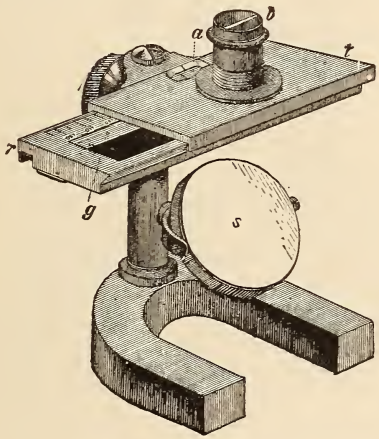


Fig. 76.—Fleischl's Hemoglobinometer.

of the blood-coloring matter. With a hemoglobin content of 20 per cent. of the normal, Dehio found an *erroneous* decline of 5.5 per cent. He recommends testing each instrument by preparing a stock solution: *i.e.*, a blood solution which corresponds exactly with the mark 100 of the hemometer. This solution gauged for the instrument to be tested is now diluted by degrees with from 10 to 90 parts of water, and with each dilution whatever differences the hemometer shows with a known percentage are recorded. In this way the sources of error may be almost wholly overcome in so far as this is possible in a colorimetric estimation.

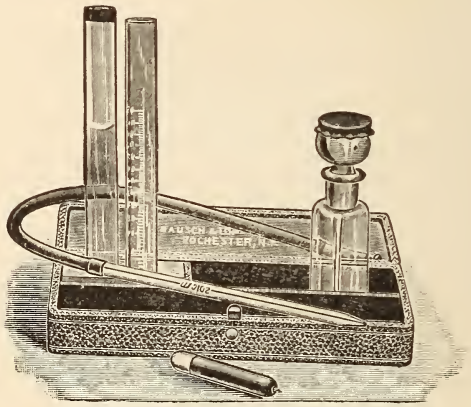
The determination of hemoglobin by **Gowers's method** is also based upon colorimetric estimation. The small apparatus[1]

[1] [To be had from the Bausch & Lomb Optical Company, Rochester, N. Y., for $2.25, while the Fleischl, made by Reichert, Vienna, costs $23.00.]

has the advantage of the Fleischl in greater simplicity, handiness, and cheapness, for which reasons the author warmly recommends it. He has used it exclusively for ten years.

It consists [see Fig. 77] of a glass tube, about eleven centimeters high, upon which is engraved a scale with 135 fine divisions, each of which indicates a volume of twenty cubic millimeters. A second sealed tube contains a "sample solution" (picrocarmin-glycerin), which corresponds to the color of 1-per-cent. solution of *normal* blood viewed in the measuring cylinder.

The accompanying capillary pipette is filled exactly up to the mark *20 cubic millimeters* with blood drawn from the finger-tip. The blood is at once mixed with a small amount of water in the measuring tube.

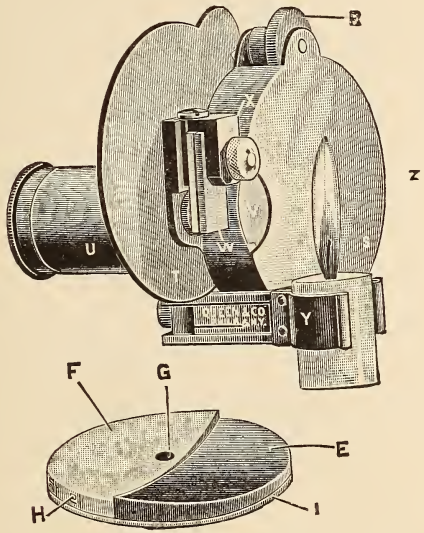


[Fig. 77.—Gowers's Hemoglobinometer.]

The latter is then viewed alongside of the "sample solution" by reflected light against a sheet of white paper in order to eliminate confusion from reflection from the tubes. Water is now carefully added, drop by drop, with a pipette, the tube being shaken, until the color of the solutions in both tubes is exactly alike. If this is obtained with a dilution reaching the 100 mark on the scale, the hemoglobin content of the blood examined is normal; if the colors correspond before this mark is reached, then the line to which the solution rises will indicate the *percentage* of normal hemoglobin present in the blood examined.

As with all colorimetric estimations, this method is also attended by certain errors, which, according to many control tests, amount to 5 per cent. For a careful performance of the test it is necessary (after quickly wiping away any blood adhering to the outside) *carefully* to empty the blood in the pipette into the water present in the graduated tube. This is done by placing the tip of the pipette just beneath the

surface of the water in the tube, blowing the column of blood out, and at once drawing fresh water into the pipette and again blowing it into the tube, in order to wash out any blood which may remain adhering to the inside of the pipette.



[Fig. 78.—Dare's Hemoglobinometer.

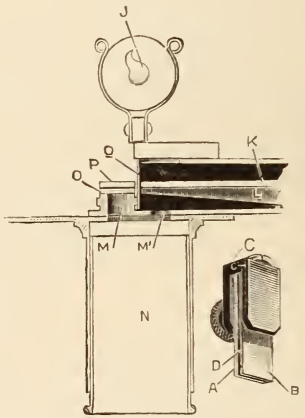
The upper figure represents the instrument ready for use (one-half actual size). *R*, Milled wheel by which the color-prism is rotated. *S*, Case inclosing color-prism, showing stage upon which the blood-pipette slides. *T*, Movable wing pivoted to case. (When drawn outward screens the eyes of observer from the light. When not in use lies superimposed upon the circular prism case.) *U*, Telescoping camera-tube in position for examination. *V*, Opening in prism case, admitting light for illumination of color-prism. (The white glass disk of prism is seen inside.) *W*, White glass of blood-pipette. *X*, Pipette clamp held in position on the stage by grooves and guides. *Y*, Detachable candleholder. *Z*, Rectangular opening in edge of case for reading hemoglobin percentage indicated by beveled blade.

The lower figure is the color-prism. *E*, Prism of colored glass. *F*, Semicircle of white glass, the edge carrying the index of hemoglobin percentage etched and filled in with black. *G*, Hole in which hub is fixed with rubber washers. (Pivots in center of case hold the prism firmly in place.) *H*, Index of hemoglobin percentage etched in black. *I*, Disk of white glass which serves the same purpose as in the blood-pipette, breaks the glare of direct light, and furnishes a white background to view the shades of color.]

[Dare's Hemoglobinometer.[1]—With this apparatus a film of *undiluted blood* is employed for comparison with a graduated

[1] Philadelphia Medical Journal, September 22, 1900.

color scale. Only a small drop of blood is required (Fig. 80) and the examination can be made in a few minutes. The pipette, composed of two rectangular plates of glass,—one white, the other clear,—which can readily be taken apart and quickly cleansed, is so adjusted as to form a chamber holding a stratum of blood of uniform thickness. The layer of blood is viewed



[Fig. 79.—Section of Dare's Hemoglobinometer.

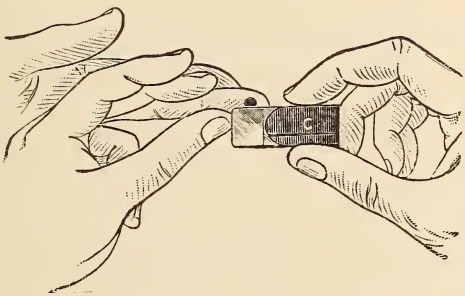
The larger figure represents a horizontal section of the instrument on a level with the center of the comparison apertures. *J*, Candle. (Curves in wick should be pointed toward instrument and junction of the springs of candleholder to insure equal illumination of blood-film and color-prism.) *K*, White glass disk of color-prism. *L*, Color-prism. *M*, Aperture through which color of blood-film is viewed. *M'*, Aperture through which the illuminated color-prism is viewed. *N*, Detachable telescoping camera-tube through which the colors, placed horizontally, side by side, are viewed. *O*, Transparent glass of pipette. *P*, White glass of pipette showing section of blood-film between *O* and *P*. *Q*, Metal septum between blood-film and color-prism.

The smaller figure shows a blood-pipette. *A*, White glass of pipette. *B*, Transparent glass of pipette. *C*, Pipette clamp, showing milled screw which makes pressure over center of white glass, preventing tilting from uneven pressure. *C'*, Groove into which corresponding guides on the stage of the instrument slide, holding the pipette firmly in position. *D*, Capillary chamber of measured thickness into which the blood is drawn automatically by capillary attraction.]

against a white background by means of a camera-tube and artificial light (see Fig. 78, *U*), preferably in a darkened room, though with certain precautions daylight does not interfere. The percentages are indicated by a graduated scale read through a rectangular opening upon the side of the instrument. Full direc-

tions accompany each apparatus. For rapidity, accuracy, and simplicity this instrument can be highly recommended.

[The **Talquist hemoglobin scale**, which has recently come into use, greatly simplifies the examination for hemoglobin. As the illustration shows (Fig. 81), it is made in book form and can easily be carried in the pocket and used at the bedside. The book contains fifty sheets of smooth filter-paper, each divided into three smaller sheets, giving a total of one hundred and fifty. It also contains a scale of ten colors for comparison. To make a test, a small sheet of the filter-paper is brought in contact with a drop of blood and the latter allowed slowly and thoroughly to be absorbed. As soon as the blood-soaked paper has lost its



[Fig. 80.—To Fill the Automatic Pipette.

Manner of filling the pipette. *C*, Under surface of pipette clamp, which is held on a horizontal plane. The filling of the pipette to its margins is observed through the transparent plate. Capillary attraction fills the pipette.]

moist gloss, it is compared by direct daylight with the colored scale. *The test cannot be made with artificial light!* The number opposite the color which corresponds to the red of the blood-soaked paper gives the percentage of hemoglobin directly (Fig. 81).

Results are obtained at once, and the errors do not exceed 10 per cent. Similar errors, however, occur with more elaborate apparatus, such as the Fleischl, Gowers, Dare, and Oliver instruments, and, therefore, this objection applies equally to them.

As each small sheet measures $2\,{}^{3}/_{4}$ by 3 inches, by using only a portion of a sheet for an examination the book can be made to serve for several hundred examinations. It is unques-

tionably the best method yet devised for the use of the practitioner.—Brooks.]

Variations in the amount of oxyhemoglobin are not rarely observed. Diminution is much more frequent than increase; the former is very common in chlorosis and severe anemic conditions, while the latter is met with only in cases in which there is coexistent increase in the number of blood-corpuscles. It may be mentioned that in addition to the pronounced increase of erythrocytes to 9,500,000 an augmentation of the hemoglobin content to 160 per cent. has several times been observed in pulmonary stenosis.

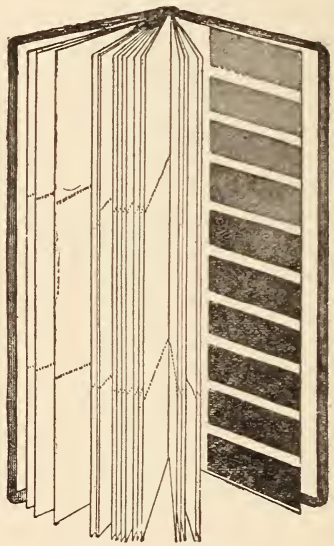


Fig. 81.—Talquist Hemoglobin Scale.

Estimation of the Dry Residue of the Blood according to Stintzing.—In general, a certain interdependent relation exists between the number of blood-corpuscles, the specific gravity, and the hemoglobin content. The parallelism is, however, not absolute. This is probably due to the fact that in the estimation of the hemoglobin the remaining proteid bodies of the blood (which, according to Bunge, amount to 7.57 per cent. in the swine) are disregarded. Therefore the estimation of the dry residue of the blood, which has recently been employed for clinical purposes (using the smallest possible amount of blood) deserves fullest consideration. Further researches in this direction are greatly needed.

Method.—From a deep puncture (running at right angles to the axis of the finger) in the finger-tip draw (if necessary, by moderate pressure upon the middle joint) 5 drops (0.2 to 0.3 gram) of blood and place in a glass dish weighing about 6 grams and provided with a tightly fitting cover. *Immediately* after withdrawal of the blood the lid is put on, and the filled dish weighed and then placed open in a drying chamber at 65° to 70° C. for about twenty-four hours. After this time the dish is quickly covered, taken from the drying chamber, and again weighed. The method of calculating is obvious.

Certain sources of error are unavoidable, but they play an unimportant rôle. (Out of one hundred and thirty-nine double estimations Stintzing found an average difference of 0.14 per cent.) It is necessary always to make two parallel estimations and to conduct the weighings as *quickly as possible* in order to as much as possible avoid evaporation of the water of the *fresh* blood and absorption of water by the dry residue.

In *healthy individuals* Stintzing and Gumprecht determined the following value for the dry residue:—

	Average.	Maximum.	Minimum.	Average Water Content.
In men	21.6	23.1	19.6	78.4 per cent.
In women	19.8	21.5	18.4	80.2 per cent.

Constant and often great diminution in dry residue occurs in chronic anemia. In chlorosis the dry residue is also reduced, but not so much as the hemoglobin. In leukemia the dry residue is relatively high in consequence of the increased number of leucocytes. Noncompensated valvular heart lesions are characterized by increased water content.

The Molecular Concentration of the Blood. Determination of the Freezing-point.—Another important peculiarity of the blood, which has recently acquired especial clinical significance, must be mentioned. Korányi was the first to establish the interesting fact that the *molecular concentration of the blood* is constant under normal conditions, but is changed in certain diseases, and almost exclusively in affections of the kidneys.

The organs which especially serve as regulators of the molecular composition of the blood and keep it constant are the kidneys, and this equalization is explained upon the theory of *osmotic pressure.* By the latter is meant, as is known, that when two solutions of different concentration are placed one over the other in strata or are separated by a permeable membrane, a *diffu-*

sion occurs, the molecules of the more concentrated solution flowing toward the less concentrated until an equalization of the molecular composition is reached.

Since the *osmotic pressure* of a solution is proportional to the number of molecules, determination of the former would give the molecular weight. In practice this is accomplished in an indirect manner. Upon the basis of the fact that the lowering of the *freezing-point* of a solvent (*e.g.,* water) by addition of foreign substances is in proportion to the osmotic pressure of the solution, a determination of the molecular weight is directly obtained by measurement of this lowering.

Determination of the freezing-point is done in Beckmann's apparatus. This consists essentially of a very delicate thermometer graduated in 100 parts, in which each Celsius degree is again divided into 100 degrees. The thermometer is placed in a glass cylinder in which the fluid to be examined is kept constantly agitated by means of a platinum stirrer. Glass cylinder, thermometer, and fluid are then placed in a freezing mixture at —4° C., and the fluid cooled under constant stirring. A moment then arrives when the fluid suddenly congeals. At the point of change from the fluid to the solid state heat is liberated, which causes the mercury column rapidly to rise to a certain point, where it remains stationary for some time,—the physical *freezing-point.* On long standing it again sinks and acquires gradually the temperature of the surrounding freezing mixture. If the freezing-point of distilled water is now determined in the same manner (the scale of Beckmann's thermometer is an arbitrary one and the 0 point is not specifically designated), and the freezing-point of the solution is subtracted from that of the water, the figure indicating how much lower the solution freezes than the water is obtained. In blood this difference is — 0.56° C. We say, therefore, in brief: The freezing-point of the blood amounts to 0.56 and an especial designation, therefore, "δ," has been selected, while "Δ" indicates the freezing-point of the urine.

The procedure in blood-examination is as follows: From a constricted vein of the arm (of course, under aseptic precautions) 15 to 20 cubic centimeters of blood are taken by means of puncture with a sharp cannula. The blood is then placed in the glass

cylinder used for freezing and defibrinated by shaking with the platinum ring, when the freezing is at once begun. In a second glass cylinder the freezing-point of distilled water is determined each time. The latter procedure we consider necessary because the position of the mercury in the U-shaped Beckmann thermometer is easily subject to variations and because the thermometers with fixed 0 point appear to me not to be reliable. After some practice the whole procedure, including venous puncture, requires about thirty minutes.

The molecular concentration of the blood in health is shown by a lowering of the freezing-point of — 0.55 to 0.57° C. On the other hand, this value undergoes an essential change—*i.e.*, the freezing-point is lower when an affection of *both* kidneys exists. The degree of difference in comparison to the normal freezing-point of the blood is in proportion to the severity of the renal affection. While in mild degrees of nephritis normal values are still found, the freezing-point becomes lower and lower with increased insufficiency of the kidneys. Lowerings to below 0.70° C. have been observed. On the other hand, the differential diagnostic and prognostic fact is to be emphasized that in unilateral renal affection, even when the latter has advanced to complete destruction of the organ, the freezing-point shows the normal height, provided the other kindney is healthy.

From this we obtain the important law that a lowering of the freezing-point of the blood below — 0.58° C. almost invariably indicates a bilateral affection of the kidneys.

(B) THE BLOOD IN DISEASE.

The alterations to be dealt with in this section are associated particularly with the corpuscular elements of the blood. Consequently the methods for the precise examination of the latter will receive detailed consideration.

A microscopic examination of the blood is often alone decisive in the diagnosis of disease. It informs us of deviations in the number, color, and size of the blood-corpuscles; of disturbances in the ratio of red and white cells, etc. It is necessary to examine both the fresh blood (see page 131) and stained dry preparations.

PREPARATION OF DRIED BLOOD SPECIMENS.

1. Spread a layer of blood as thinly and uniformly as possible upon a cover-glass [or slide]. It is first absolutely necessary to employ only thoroughly cleansed cover-glasses [or slides] free from grease, which have been washed preferably in absolute [or 95-per-cent.] alcohol or dilute nitric acid, and polished with soft *leather* [or clean, unstarched linen, silk, or cotton cloth]. The glasses should be grasped with forceps; if the fingers are used the warm air given off from them will favor disturbing alterations in form, which occur readily enough even without such agency. For the same reason breathing upon the preparation should be avoided. A small drop of blood drawn from the finger-tip or lobe of the ear is taken up with a cover-glass [or slide], another glass placed carefully upon it without pressure, and the two quickly drawn apart. Or the drop of blood may be taken up on the edge of a cover-glass or smoothly ground slide and quickly spread into a uniform, thin layer upon another cover-glass or slide.

The spreading can also be done with an especially constructed mica spatula, or a fine camel's-hair brush, by dipping these instruments in the blood and then stroking the surface of the slide or cover-glass. [Excellent smears can be made by dipping the end of a strip of cigarette paper in the blood and then drawing it quickly across the surface of a slide or cover-glass.—BROOKS.]

All of these methods will yield the result desired; it is a matter of little importance which is used. The main thing is the preparation of a delicate layer of blood in which the cells are isolated, and *not superimposed*. To secure such spreads requires great care and no little practice.

2. After the preparations have been thoroughly dried in the air they are subjected to further *fixation*. If it is desired to make an examination as quickly as possible, *fixation by heat for a few minutes at about from 110° to 115° C., or in a mixture of absolute alcohol and anhydrous ether* [*equal parts*] *or, finally, in absolute alcohol alone* may be employed. In the latter the preparations should remain for at least half an hour, or, better, one hour. *Formol,* a mixture of 40-per-cent. formaldehyd in *methyl* [wood] alcohol and water, "fixes" in one minute.

One part of formol is first diluted with 10 parts of water, and of this mixture, again, 1 part is diluted with 10 parts by volume of alcohol. In this solution the dried blood preparations are faultlessly "fixed" in one minute. They can subsequently be subjected to most staining reagents without alteration of either the form or composition of the elements. The protoplasm as well as the hemoglobin is transformed into an insoluble and fixed condition, and the properties of the cells for the reception and retention of stains are wholly preserved.

STAINING OF DRIED BLOOD PREPARATIONS.[1]

1. Stain with 0.1- to 0.5-per-cent. watery solution of eosin upon which the cover-glass preparation should float for from ten to twenty minutes. Wash in water, dry, and imbed in xylol-Canada balsam. Staining is greatly hastened by heating the solution. Alcoholic solution of eosin (0.25 to 0.5 per cent.) stains the preparations in from one-half to one minute. The red blood-cells are stained a bright and uniform red. The protoplasm of the colorless [white] corpuscles is only slightly tinged. The eosinophilic granules (which see) appear as intensely stained minute spherules.

2. Stain with Ehrlich's hematoxylin-eosin solution for from twelve to twenty-four hours (Plate IX, Fig. 15). The solution is prepared as follows:—

Distilled water,	
Alcohol,	
Glycerin	of each 100.0
Hematoxylin	4.0 to 5.0
Glacial acetic acid	20.0
Alum in excess.	

The fresh solution should stand for from four to six weeks in the sun; then about 1 per cent. of eosin is added. After twenty-four hours' staining (which is best done in a closed dish exposed to sunlight) and thorough washing in water, drying, and imbedding in balsam, the red blood-corpuscles appear strawberry red (now and then with a slight tinge of orange); the nuclei, when present, deep black; the cell-body of the leucocytes a light and their nuclei a dark lilac; the eosinophilic granules intensely red; and the small lymphocyte nuclei usually blackish—a shade lighter than those of the erythrocytes, their protoplasm scarcely tinged.

3. Stain with **Ehrlich's tri-acid solution** (Plate IX, Fig. 14), which is made as follows:—

Distilled water	100.0
Orange G	135.0
Acid fuchsin	65.0
Distilled water	100.0
Absolute alcohol	100.0
Methyl-green	125.0
Distilled water	100.0
Absolute alcohol	100.0
Glycerin	100.0

These are to be gradually mixed with each other. The mixture can be used only after long standing [one or two weeks—Brooks]. The

[1] Grübler's stains, made in Leipzig, are the best.

preparations are floated, according to Ehrlich, for only two minutes—in the author's opinion, six to eight minutes would be better—upon the solution. They are then washed in plenty of water, dried, and imbedded in xylol-balsam. The erythrocytes are stained yellow, their nuclei greenish blue. The majority of the leucocytes show fine, violet neutrophilic granules and a greenish-blue nucleus. The eosinophilic granules are stained bright red.

This method of staining is highly recommended for the rapidity with which it can be conducted. The fine differentiation, however, cannot always be brought out with a solution prepared according to the above formula. Apparently, the discoverer of this staining mixture has himself often had such experiences, for the formula given above, which the author obtained directly from his colleague, Ehrlich, differs greatly from others published by him.

4. Ehrlich's tri-acid solution has been modified by Aronson and Philip as follows:—

Saturated watery solutions of orange G extra, acid rubin extra, and crystallized methyl-green are first prepared. After these solutions have cleared by sedimentation, the composition is as follows:—

Orange solution	55 c. cms.
Acid rubin solution	50 "
Distilled water	100 "
Alcohol	50 "

Then add:—

Methyl-green solution	65 c. cms.
Distilled water	50 "
Alcohol	12 "

The mixture must stand for from one to two weeks.

One drop of this solution in a Petri dish of water suffices distinctly to stain the preparation in twenty-four hours. Before imbedding, the preparations are washed in water, briefly in absolute alcohol, cleared in origanum-oil, and imbedded in xylol-Canada balsam.

The shades of color correspond to those objects stained with tri-acid. Rieder highly praises this stain. The author, however, believes Ehrlich's tri-acid solution is much superior.

5. Staining with **Chenzinsky-Plehn solution**:—

Concentrated watery solution of methylene blue	40 grams.
0.5-per-cent. (prepared in 70° alcohol) eosin solution	20 "
Distilled water	40 "

Stain the preparation for twenty-four hours, if necessary, in the incubator. The *heated* solution stains in fifteen minutes, but not so delicately. The red blood-cells are stained eosin-red; the eosinophilic granules, bright red; the nuclei, blue (Plate IX, Fig. 14).

Staining successively with alcoholic solution of eosin and concentrated watery solution of methylene blue also gives sharp pictures. For example, staining for from two to three minutes in heated 0.5-percent. alcoholic solution of eosin and the same length of time with a saturated watery solution of methylene blue.

[The following method will be found useful for hasty examinations: By means of a pipette, drop upon the previously fixed (wood alcohol) specimen a small quantity of:—

Saturated alcoholic solution of eosin ..... 1 part;
Water ..... 10 parts;

and *heat* over a free alcohol or Bunsen flame until vapor arises; wash in water and stain for half a minute with *cold* Löffler methylene blue solution; wash in water, dry thoroughly (absorbent paper and flame), and examine in cedar-oil with $^1/_{12}$ oil-immersion lens, without cover-glass. This method can be used in examining blood microscopically for any changes. To avoid overstaining with the methylene blue solution, it is advisable to examine the preparation, **just after the blue has been washed off and while it is still wet,** with a $^2/_3$ objective. In a well-stained specimen the red corpuscles should be pale pink or light red, and the white-corpuscle nuclei very pale blue. If the red corpuscles have a purplish or amethyst hue, the blue dye has acted too long or too intensely. In this case restain with eosin and proceed as before, allowing the blue to remain a shorter time on the specimen. Sometimes the blue will overstain no matter how much care is observed. This can be overcome by diluting the Löffler solution with one-half its bulk or more of water. If the white-corpuscle nuclei are not stained after the time above mentioned, restain with the blue until the nuclei take on the pale-blue tinge. When the right coloring of both corpuscles is attained (as can readily be seen by examining with a $^2/_3$ objective as above described), dry off the water and examine. With a little care, good results can be secured.

The **malarial organism** can also be demonstrated by this method. It appears blue within the pink-stained bodies of the red blood-corpuscles. The nuclei of the white blood-corpuscles, as has already been said, are also stained blue, and the eosinophile granules, within the protoplasm of the white corpuscles, bright red.

My associate, Dr. B. F. Cline, has devised a very simple and excellent method of staining applicable for ordinary blood examinations in which differential staining of the leucocyte granules is not essential. It is as follows:—

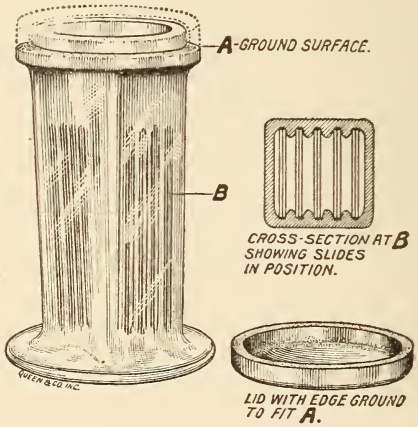
The blood is spread in the ordinary manner upon a slide and allowed to dry in the air. The preparation is then *gently heated* (not made hot!) about six inches above a free gas or alcohol flame, and when cooled is placed for two minutes in methyl *(wood)* alcohol containing 0.5

per cent. of eosin. Wash in running water and stain for two minutes in Böhmer's hematoxylin, which is made as follows:—

I.	Hematoxylin crystals	1 gram.
	Alcohol (ethyl)	25 c. cms.
II.	Potash alum	10 grams.
	Distilled water	200 c. cms.

Heat in water-bath until the alum crystals are dissolved.

When I and II are dissolved, mix the two solutions and let stand in open, wide-mouthed bottle for from eight to ten days; then filter. Finally, as a preservative, add 2 or 3 small lumps of gum-camphor. Use full strength.



[Fig. 82.—Copland Staining Dish.]

After staining with this solution, wash in running water, dry in flame, and examine without cover-glass, placing the immersion oil directly upon the preparation.

With this method the leucocyte nuclei stain a deep blue and are very distinct and sharp; the red cells are colored a dark pink or light red (see Plate IX, Fig. 15). In differential counts in leucocytosis the method is all that can be desired. The malarial organism is also well stained.

It is convenient to keep the eosin and hematoxylin solutions in salt-mouth bottles holding about four ounces. In this way the prepared slides can be placed upright in the stains and precipitates are avoided. The Copland staining dish (Fig. 82) is much better adapted to this purpose. The solutions hold their staining properties for months.—Brooks.]

Besides Ehrlich's tri-acid solution, the author would recommend Chenzinsky-Plehn staining mixture for the practitioner's

use in preference to all others; it is adapted for the examination of constitutional blood affections as well as for the detection of the various parasites occurring in the blood.

[Leishman's Modification of Romanowsky's Stain for Malarial and Other Blood-films.[1]—This method is so easy of application and requires so little time and skill in its manipulation that it may advantageously be employed for ordinary blood-staining in preference to any procedure now in use. It gives excellent results also with "smear" preparations from bone-marrow, the spleen, liver, and "cancer" juice. Bacteria stain by this method with great distinctness. No previous fixation is necessary, because the staining solution fixes and stains at the same time.

SOLUTION A.

Medicinal methylene blue (Grübler)	1.0
Distilled water	100.0
Sodium carbonate	0.5

Heat in a paraffin oven for twelve hours, and then allow to stand at room temperature for ten days before use.

SOLUTION B.

Eosin, extra B. A. (Grübler)	1.0
Distilled water	1000.0

Equal volumes of Solutions A and B are mixed in a large open vessel and allowed to stand for from six to twelve hours, stirring from time to time with a glass rod. The abundant flocculent precipitate which forms is collected on a filter and thoroughly washed with distilled water until the filtrate is colorless or pale blue. The insoluble residue is now carefully collected, dried, and powdered. The powder thus obtained has a greenish, metallic luster and contains the active staining ingredient present in Romanowsky's stain.

Dissolve 0.15 gram of the powder in 100 cubic centimeters of methyl alcohol (Merck's "for analysis") and preserve in glass-stoppered bottle until used. The solution does not deteriorate with age.

Staining.—Films are made in the ordinary way and allowed to dry in the air. The thinner and more uniformly the blood is smeared, the better the result will be. Three or 4 drops of the stain are allowed to act for about half a minute and then twice the amount (*i.e.*, from 6 to 8 drops) of distilled water is added and allowed to mix with the dye. After from five to ten minutes, according to the thickness of the smear and the character of the film, wash in distilled water, *allowing a few drops of water to remain on the preparation for one minute.* Dry in the air and examine in oil with $^1/_{12}$ immersion lens. Allowing the stained preparation to be acted upon by the distilled water for a minute after staining "intensifies the staining, removes the remains of the deposit, and changes the tint of the erythrocytes from greenish blue to a transparent pink."

[1] British Medical Journal, September 21, 1901, page 757.

According to Leishman, the *appearances of the blood-films* stained by this method are as follows:—

Red Blood-corpuscles. — Pale pink or greenish in tinge; semi-transparent.

Polynuclear Leucocytes. — Nuclear network stained a deep ruby-red color, with sharply defined margins. Extranuclear protoplasm, colorless. Fine eosinophile granules, red.

Mononuclears.—Nuclei, ruby-red, with extremely sharp, clear outlines. Extranuclear protoplasm, pale *eau-de-Nil* or blue, occasionally showing a few red granules.

Lymphocytes.—The same as mononuclears, except that the nuclei are, as a rule, more deeply stained.

Coarse-grained Eosinophiles. — Nucleus, ruby-red, but not so densely stained. Granules, pale pink.

Basophiles.—Granules very densely stained, of a deep purplish black tint. Nucleus, red, but usually more or less masked by granules overlying it.

Nucleated Red Cells.—Nucleus, almost black, with sharp outline; extranuclear portion, gray.

Blood-plates.—Deep ruby-red, with spiky margins, frequently showing a pale-blue peripheral zone surrounding the red center.

Bacilli and Micrococci.—Speaking generally, these stain evenly blue, but, by prolonging the period of staining and subsequently decoloring with absolute alcohol, many interesting differences may be noted with different organisms, by which structural details are brought out not generally observed by other staining methods.

Malarial Parasites.—The body of the parasite stains blue and its chromatin ruby-red; in the case of the tertian parasite Schüffner's dots are well marked in the containing red blood-corpuscle.

This method is superior to that of Jenner. Jenner's solution soon loses its staining properties, and even when fresh the results are not always satisfactory. Wright's and Goldhorn's stains offer no advantages over the method here described, except, perhaps, in the saving of time. We have not space to more than refer to these stains.—Brooks.]

CHANGES IN THE BLOOD IN DISEASE.

(A) General Summary.

As compared with what was obtained by earlier study of fresh blood preparations, our knowledge of the changes occurring in the red and white blood cells in disease has been greatly extended by the application of analytical staining methods. Whenever possible, every examination of the blood should be conducted upon fresh as well as upon hardened and stained preparations.

The following deviations have thus far been recognized:—

I. In the Red Cells.

1. In fresh preparations the red cells not infrequently show decided **changes of form,** which, at Quincke's suggestion, are classed under the term **poikilocytosis** (ποικίλος = variegated) (Fig. 83). The erythrocytes are drawn out into pear- or flask-shape; or are provided with a long pediclelike prolongation; or they appear anvil-, wallet-, or posthorn- shaped; or resemble a cross or star. While some are extremely small, others appear to be unusually large, and sometimes show a wavy, serrated contour.

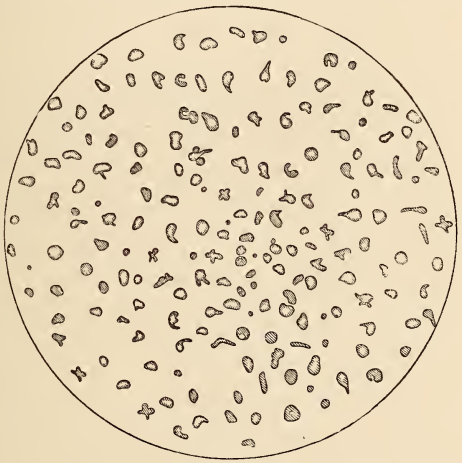


Fig. 83.—Poikilocytosis.

All, even the smallest, elements show distinct *umbilication.* They are without doubt to be looked upon as segmentation bodies derived from old erythrocytes by processes of constriction. (In accordance with this view, Ehrlich designates them as "schistocytes [σχιστός = cleft, from σχιζειν = to cleave]). Hayem alone considers them transition forms of his hematoblasts (Bizzozero's blood-plates) to blood-cells, although he had never observed their actual transformation into a colored blood-corpuscle.

As poikilocytosis is comparatively most frequently associated with profound diminution in the number of red blood-corpuscles, it may, as some observers have suggested, possibly be a conservative process for increasing the respiratory surface of the blood-corpuscles.

2. Furthermore, the **appearance of nucleated red blood-corpuscles** is deserving of attention. These occur in two forms: as *normoblasts,* which are small, nucleated, colored bodies the size of a normal red blood-corpuscle; and as *megalo-* or *gigantoblasts*—that is, nucleated erythrocytes, which are from three to five times larger than ordinary red blood-cells (Plates VIII and IX, Figs. 12 to 16).

The nuclei of the normoblasts, which are not infrequently observed to be segmenting, are generally centrally located, sometimes peripherally. They are characterized by the intensity with which they stain; the nuclei of the megaloblasts are stained somewhat paler, but always deeper than the nuclei of the white blood-corpuscles.

On staining with hematoxylin-eosin the nuclei appear as homogeneous or coarsely granular, uniformly black objects and generally present a sharply defined contour. With the tri-acid stain they appear as intense greenish-blue or dark peacock-blue bodies in sharp contrast with the yellow-stained stroma of the red blood-cells. With Chenzinsky's stain they are distinguished by a dark-blue or blackish-blue color.

Except in the newborn, nucleated red blood-corpuscles are never met with in *normal* blood. They are of frequent occurrence, however, in pathologically altered blood, especially in pernicious anemia and leukemia. They are less often observed in severe acute anemias after loss of blood and in chlorosis.

Bodies very closely resembling in color the stained nuclei of normoblasts are often found free without any inclosing stroma. This phenomenon and the fact that the peripherally located nuclei of the normoblasts sometimes show only a small area of contact with the cell-body speak in favor of the view that the nuclei of the normoblasts may be extruded.

Ehrlich sees in this a significant indication of regeneration in so far as he believes these nuclei again take on protoplasm (?). It is different with the *megaloblasts,* which always contain only *one* nucleus, which, as has already been mentioned, stains less intensely and is said not to be extruded. The normoblasts occur in the normal bone-marrow of adults, the megaloblasts only in that of the embryo. This seems to indicate that the appearance of the latter in the blood of patients marks a "reversion to the embryonic" state. Their exclusive appearance always points to severe pernicious forms of anemia.

3. The occurrence of *abnormally large, nonnucleated* erythrocytes is of the greatest diagnostic value. These are designated as *megalocytes,* or *giant blood-corpuscles.* They are from 10 to 14 μ in size, and show a very variable hemoglobin content. Their diagnostic significance in pernicious forms of anemia has been emphasized especially by Laache, with whose views the author is entirely in accord. In rare instances, and then only sparingly, these abnormally large red blood-cells are observed in severe *chlorosis.*

4. Of less significance is the occurrence of very small, round bodies from 2 to 5 μ large, resembling the blood-plates, which, because of their distinct hemoglobin content, are to be interpreted as erythrocytes, although they are not umbilicated. These are called *dwarf blood-corpuscles,* or *microcytes.* According to Gram, they are newly formed red blood-cells, since they most frequently occur shortly after severe acute loss of blood (Plate IX, Fig. 13, *b* and *c*).

5. Not infrequently the so-called **anemic** or **polychromatophilic degeneration** of the red blood-disks is met with. This is manifested on staining with eosin-hematoxylin or eosin-methylene blue by the absence of homogeneous hemoglobin staining and the appearance of a "washed-out" violet shade of color, because the degenerative changes of the stroma favor action of the nuclear dyes.

It is quite probable that the question here is one of death of older forms, albeit the same staining phenomenon is sometimes also observed in *nucleated* corpuscles (Plate IX, Fig. 15, a_2), which may justly be considered as young or antecedent forms of the red blood-corpuscles. Here, however, the severity of the collective features of disease points to the pernicious character of the disturbances, the expression of which is the continuation of the degeneration to the fresh elements introduced from the bone-marrow. It appears to me probable that the polychromatophilia of the blood-corpuscles should not be considered as a degeneration, that these formations are rather to be looked upon as elements in an early stage of development and that their appearance is to be interpreted as an expression of forced regeneration. The statements of C. S. Engel, who saw these questionable formations in embryonic blood, seem to confirm this view; and, secondly, I would add that polychromatophilic blood-corpuscles are very frequently met with in malarial anemia, which always is referable to destruction of individual erythrocytes attacked by parasites, in which, however, the question is not one of a noxa which injures

all blood-corpuscles alike. Those erythrocytes which contain a parasite and have already been more or less destroyed—*i.e.*, are undergoing degeneration—are particularly marked by pale staining.

6. Still less understood are the staining alterations of red blood-cells which Ehrlich designates as "*stippled*" and are characterized by the appearance of very numerous, densely arranged blue puncta on staining with methylene blue mixtures. They are not infrequently found in anemia due to various causes. Contrary to Plehn, they are in no way associated with malarial parasites.

II. In the Leucocytes.

Long ago Max Schultze, Virchow, Erb, and others described different forms of leucocytes. The purely morphologic characters, their differences in size, the more or less coarse granulation, and the variable shape of the nucleus offered a basis for differentiation. In regard to origin, also, certain notions had been entertained; for example, according to Virchow, the small mononucleated forms are derived from the lymph-glands, the large mononucleated from the spleen.

As a result of Ehrlich's analytic researches by staining as applied to the study of the leucocytes, the following has to a certain degree been established:—

Aside from the differentiation into small and large mononucleated, and fine and coarsely granular multinucleated cells, according to the number of nuclei and size of the cell, the microchemic differences of the granulations permit the following subdivisions:—

1. Eosinophilic cells (Plate IX, Fig. 13, *d*).

In fresh, unstained preparations these can be recognized by the strongly refractive, coarse granulation of the contractile protoplasm (Fig. 71, *e*). Since, however, they may be mistaken for basophilic cells, which will later be described, they can be positively identified only in stained preparations.

The eosinophilic α-granules are characterized by their peculiar affinity for the *acid* anilin dyes. They take up the dye with extreme readiness. When stained with eosin they appear as bright-red spherules filling the body of the cell, to which they give the appearance of a strawberry. Eosin, aurantia, and nigrosin stain the granules in concentrated glycerin solution (after a number of hours); fluorescin, ammonium picrate, and orange stain them *only* in *watery* solution.

The suspicion readily aroused in examination of fresh specimens that these granula consist of fat is disproved by their resistance to the action of absolute alcohol, ether, and carbon bisulphid. Furthermore,

they are soluble in water and glycerin, and are not stained by osmic acid. Finally, the view that they are droplets of hemoglobin cannot be entertained, since it has been shown that on staining dry blood preparations with a 5-per-cent. solution of carbolic acid saturated with eosin and ammonium picrate the red (hemoglobin) blood-cells are stained pure yellow and the eosinophilic granules bright red.

The eosinophilic cells of *normal* blood are always the size of the ordinary (multinucleated) leucocytes, and when viewed upon a warm stage manifest active ameboid movements. The numeric ratio to the remaining leucocytes varies between wide limits in different perfectly healthy individuals as well as in one and the same person at different times. Whether the origin of the eosinophilic cells is in the bone-marrow (Ehrlich) or not is yet to be decided. The eosinophilic cells found in the bone-marrow are, for the most part, very different from those occurring in the circulating blood of healthy persons, not only in size, but also in the form of the nucleus. *On the other hand, gradual development of finely granular cells into coarsely granular* (i.e., *eosinophilic) cells as well as division of eosinophilic cells can unquestionably be observed in the blood.*

It is not increase in the number of (normal) eosinophilic cells, but the occurrence of such eosinophilic forms as are numerous in the bone-marrow and usually absent from the blood which is of chief diagnostic importance. These will receive special consideration in the description of the findings in leukemic blood.

The statements in regard to diminution or increase of the eosinophilic cells in the blood demonstrates that this phenomenon is of quite irregular occurrence. It seems certain, however, that in severe anemia and most acute infectious diseases the number of (normal) eosinophilic cells is *slight.*

2. Neutrophilic cells (Plate IX, Figs. 14 and 15).

Those granules which assume a characteristic violet hue when stained with the tri-acid mixture are designated according to Ehrlich as neutrophile (ϵ-) granulations.

The leucocytes with neutrophilic granules usually show peculiarly shaped nuclear figures in the form of an S, V, F, M, etc., or several distinct nuclei. They constitute by far the greater number of multinucleated leucocytes present in the blood, and are without much doubt derived from the mononucleated cells.

According to Ehrlich, all *pus-cells* manifest the characteristic violet granulations (see eosinophile cells in gonorrhea, page 47).

While the eosinophilic and basophilic granules, which will next be considered, were observed by Ehrlich in all animals, the neutrophilic granules, the origin of which is to be sought in the bone-marrow and spleen, appear to occur *only in man.*

3. Basophilic (Mastzell) Granules (Ehrlich's γ- and δ-granulations) are deeply stained with *basic* anilin dyes, among others with a concentrated watery solution of methylene blue, which is allowed to act for from five to ten minutes.

Ehrlich gives the following formula for staining the mast-cells (γ-granulations):—

Water	100 c. cms.
Saturated absolute alcoholic solution of dahlia	50 "

To the clear solution obtained after standing are added from 10 to 12.5 cubic centimeters of glacial acetic acid. Aside from bacteria, the solution stains *only* mast-cells (red), while pus-cells are barely tinged.

The basophilic granules are usually not so densely arranged in the protoplasm as in the first-named granulations; the γ-granula approach in size the eosinophilic granules; the finer are designated as δ-granulations. As a rule, the granula of the individual cells are not of uniform size.

Recent investigations have shown that occasional basophilic cells can be found even in perfectly healthy individuals. The author frequently met them in *large numbers* in the sputum of asthmatics (see page 202). According to Ehrlich, they are derived from the fixed connective-tissue cells, and partly also from the bone-marrow.

As to the **percentage** of the different forms of leucocytes occurring in *normal* blood, the following has been determined:—

Among enumerated leucocytes there will usually be found about 25 per cent. of small mononucleated cells,—lymphocytes,—while the remaining 75 per cent. are multinucleated cells of larger size. Of these, about from 2 to 4 per cent. are eosinophiles and barely $^1/_2$ per cent. are basophiles. All other multinucleated forms show neutrophilic granules.

Ehrlich has repeatedly very strongly emphasized that "a single cell never contains two kinds of granulations." Accord-

ing to the author's experience, this statement must be accepted with limitations (see "Leukemia"). At any time one can convince himself that, for example, the cells of gonorrheal pus (Ehrlich's paradigma) when stained simply with eosin often show the same fine granulations as on staining with tri-acid. Consequently, the author cannot accord to the neutrophilic granulations the high degree of significance which Ehrlich and his pupils have assigned to them. It is different, however, with the eosinophilic granules. There is scarcely a doubt that we have here to deal with a very remarkable specific reaction. The size and intensely refractive character of the unstained granules, but especially the rapid and intense staining with acid anilin solutions, serve sharply to distinguish them. However, certain cells are sometimes met with which, in addition to small, scarcely visible, faintly tinged granules, contain unquestionable coarse eosinophilic granulations. These cells may justly be looked upon as transitional forms. Max Schultze long ago drew attention to the fact that transition from fine to coarsely granular cells can be observed upon the warm stage. Here, however, it must be assumed that cells containing fine—*i.e.*, neutrophilic—granulations also contain eosinophilic granula.

Of what significance are these granulations? At the present time it is impossible to answer this question with any degree of certainty. Ehrlich considers the granules simply as secretion products of the cells; Altman considers them true elementary organisms (bioblasts) which, by their aggregation, form the cell, nucleus, and protoplasm, and play an active and very important rôle in the life of the cell. In support of his theory Altman cites the observations upon the absorption and secretion of fat. These observations have shown that the "bioblasts" gradually become loaded with fat, and then can be demonstrated with osmic acid as delicate, gray or blackish rings or black globules. It is very probable that, for example, the fat is taken up by the granules in soluble form and transformed by synthesis into neutral fat. Years of study will be necessary for a solution of the problem. For the present, however, the various kinds of granulations may be regarded as possessing a certain degree of semeiotic importance in clinical diagnosis.

(B) In Special Affections.

Having sketched in a general way in the previous section the methods of examination and the alterations to be observed in the blood, we will now describe the special microscopic features

to be seen in individual diseases. To simplify matters the description of the remaining characteristics of the blood will be included here.

The Anemias.

These may be divided into the following groups:—

Simple primary anemia. Simple secondary anemia.
Severe pernicious anemia.

1. In **chlorosis**, or **simple primary anemia**, the blood is often macroscopically distinctly paler; the hemoglobin reduced to 50, 40, and even 15 per cent.; and the specific gravity not rarely pronouncedly reduced,—on an average, 1.040. *As a rule,* the number of erythrocytes is normal and the form is usually unaltered. The white blood-cells are not increased; now and then the ratio of eosinophiles is increased.

Severe cases of chlorosis, however, manifest many deviations. The number of red blood-disks may fall to from 3.5 to 2.4 millions, the *hemoglobin content* to 20 or 15 per cent. (!), and consequently the *specific gravity* to from 1.033 to 1.028. Chief of all, however, the red blood-corpuscles present such changes in form as fully to justify us in speaking of *intense poikilocytosis* (Fig. 83). Its occurrence in chlorosis is often disputed; the "variegated" forms are said to be produced as a result of "evaporation," to which chlorotic blood is soon disposed. The author would oppose the acceptance of this objection,—which of itself concedes an alteration of the blood,—because poikilocytosis is met with in fresh blood drawn under exclusion of air as well as in dried preparations. It should be remembered, however, that this phenomenon *usually* occurs only in the severest forms of chlorosis, in which a tendency to thrombosis exists and dangerous accidents—embolism of the pulmonary artery and the like—may occur. Consequently its occurrence must be interpreted as a warning of the necessity for a guarded prognosis. In but one instance the author met with numerous "variegated" forms in a case of chlorosis in which the general subjective and objective condition was in no way gravely disturbed. Gräber and Gram saw poikilocytosis relatively frequently, and they, as well as Laache, found that the diameter of the erythrocytes was not rarely reduced. On several occasions

the author also has found nucleated red blood-corpuscles in severe chlorosis; quite frequently a few abnormally large nonnucleated red blood-cells are met with.

2. In **simple secondary anemias** the alterations in the blood are, as a rule, dependent upon the nature and duration of the primary affection (phthisis, carcinoma, syphilis, chronic nephritis, malaria, etc.). There is almost always a more or less intense *diminution in the number of the erythrocytes* and a *corresponding* reduction in the amount of hemoglobin. The number of leucocytes is *not* reduced; on the contrary, they are often very considerably increased.

Usually the form of the red blood-corpuscles is unaltered; there are no variations in size worthy of mention. Now and then, however, decided poikilocytosis and the occurrence of nucleated red blood-cells are observed. If in such cases the number of erythrocytes is very greatly reduced, it is a question whether the diagnosis of simple secondary anemia should not be rejected. In these doubtful cases the numeric ratio of the leucocytes as well as the species of nucleated red cells present is of importance. If the former are increased and the latter predominating in the size corresponding to that of normal red blood-cells, then the existence of a secondary form is usually to be assumed.

Transition into the chronic pernicious form is rare, but it unquestionably occurs.

3. **Progressive pernicious anemia** (Plate IX, Fig. 13).

Since 1868, when Biermer gave a masterly description of this disease, the diagnosis of this peculiar affection is generally made without great difficulty. The intense paleness of the external skin and mucous membranes, increasing weakness, gastric disturbances, cutaneous and retinal hemorrhages, fever, etc., in addition to the blood-findings, usually render the diagnosis certain. But even for the experienced observer who has seen a few dozen cases confirmed at necropsy, it is not rarely difficult to distinguish between an *essential* progressive pernicious anemia and a form dependent upon a malignant neoplasm or other serious disease. If a malignant growth exists a solution of the problem is of little avail; it is otherwise when the threatening anemia is dependent upon visceral parasites (bothriocephalus,

anchylostomum, etc.) or syphilis, since here a cure can be obtained after removal of the cause. Unfortunately, our present methods of blood examination do not suffice to enable one in doubtful cases to make this important differential diagnosis from the blood-findings. Points worthy of attention in this connection are given in the following:—

Among contributory causes, repeated small hemorrhages (uterine myomata), less often a single profuse hemorrhage, dyspeptic disturbances, intestinal parasites, pregnancy, and parturition; finally, chronic infectious diseases, such as dysentery, malaria, syphilis; less often acute diseases, such as typhoid fever, play a more or less obvious rôle.

In addition to severe degenerative changes in the liver, heart, and kidneys, and the very rarely absent transformation of the fatty marrow into red bone-marrow, autopsy reveals a most interesting lesion, first recognized by Quincke, namely: a large deposition of iron in the liver and more or less intense *pigmentation* (pigment infarcts) in the liver, spleen, and kidneys (Eichhorst, Hunter, Birch-Hirschfeld). The latter phenomena point directly to great destruction of red blood-corpuscles, as may be perceived even *intra vitam* on examination of the blood.

Blood-findings.—The blood is normal in color or strikingly pale, but sometimes darker than normal, like weak coffee, or even tar colored. It is often very thin; so that it does not spread into a thin layer and dry upon a cover-glass so well.

The number of *erythrocytes* is always diminished, often to an astonishing degree. Figures of from 400,000 to 800,000 are by no means rare. Quincke counted in one case only 143,000 in a cubic millimeter, and the author himself found 375,000 as the minimum limit. On the other hand, the number of *leucocytes* remains normal, an increase being very rare.

The total hemoglobin content is reduced to 15 or even 12 per cent., but that of the individual corpuscles is not; indeed, it is not rarely increased in the latter, as is at once apparent by comparison of the figures giving in percentage the diminution in the number of erythrocytes and hemoglobin. For example, the number of red blood-corpuscles may be as low as 16 per cent., while the hemoglobin content is only as low as 20 per cent.

Microscopic examination generally shows an *enormous poikilocytosis* [see page 157], slight tendency to the formation of rouleaux, frequent microcytes and strikingly numerous mega-

locytes, and usually, also, nucleated red blood-cells. Very frequently the cells are imbedded in a pale, homogeneous (albumin?) layer, such as is otherwise not observed in stained dried blood preparations.

In the **microscopic** diagnosis of pernicious anemia the author considers the *excessive occurrence of abnormally large* red blood-corpuscles of chief importance.

A point of importance in the differential diagnosis between essential anemia and that form secondary to a malignant neoplasm is that, in the latter, the multinucleated leucocytes are in the *majority* of instances increased, while in the former they are rather diminished. There are exceptions, however.

Hayem always found the *blood-plates* greatly reduced in number, and often entirely absent. If this is found to be the rule, it might offer an explanation for the constant *reduced tendency of the blood of pernicious anemia to coagulate.* In a rapidly progressive typic case in which attention was directed to this particular point, the author did not receive the impression that the blood-plates were strikingly diminished. However, as counting of the blood-plates cannot be done with any degree of exactness, the results obtained here must be based more or less upon estimated values.

For **staining** dry preparations, Ehrlich's tri-acid and eosin and methylene blue solutions are recommended in preference to all others.

1. In the preparations stained for from six to eight minutes with the *tri-acid* solution the red blood-cells appear dark yellow, the nuclei of the nucleated red cells green or peacock-blue. The leucocytes, chiefly polynuclear, have a distinct violet granulation. The eosinophiles are, on an average, 12 to 14 μ in size. Isolated large, mononucleated leucocytes with unquestionable, densely arranged neutrophile granules also occur, while others show a pale protoplasm. The nuclei of the leucocytes are pale blue. Many red blood-cells are pale; others show a faded reddish blue tinge.

2. On staining with *eosin-hematoxylin*, preferably for from twenty to twenty-four hours, the red blood-disks are strawberry-red, with a slight shading of orange, and the nuclei are blackish. Megalocytes and megaloblasts up to 19.5 μ in size could be observed by the author. The leucocytes, which are seldom—and then only slightly—increased, show upon a bright lilac-colored body dark lilac-colored nuclei, which, especially in the large mononucleated cells, present a distinct reticulated structure, the light spaces in which may be interpreted as vacuoles, or Altman's nuclear granula. *The nuclei of the lymphocytes are much darker, approaching the shade of color of the normoblast nuclei; so that when the protoplasmic periphery shows a uniform and strawberry color it may*

sometimes be doubtful whether the bodies seen are lymphocytes or extruded erythrocyte nuclei. In many red blood-corpuscles a more or less distinctly bluish-red tone of color of the whole cell-body can be seen.

Chenzinsky's stain also gives good results.

Nucleated red blood-corpuscles, often of unusually large size, occur either in small or in large numbers, and are but rarely absent (see page 158). According to the author's experience, anemic degeneration is rare. The behavior of the *eosinophiles* is wholly uncharacteristic; diminution as well as slight increase may be observed; complete absence appears to be a very unfavorable prognostic sign.

Nucleated red blood-cells may occur in *all* severe anemias. Ehrlich ascribes an important rôle to their size; according to him, the presence of *normo*blasts permits a more favorable prognosis, since they are to be interpreted as an indication of an increased production of normal elements from the bone-marrow. The prominent part played by the bone-marrow in the formation of blood has been positively determined by Neumann and Bizzozero. In severe anemias there is found a more or less advanced transformation of the yellow, fat bone-marrow into red bone-marrow. In this way an increased regeneration of the more readily destroyed red blood-cells is secured. From this view of the question it might be assumed that the absence of *nucleated* red blood-cells is an indication of the *nonoccurrence* of the significant transformation into red marrow. This, however, is *not* in harmony with *facts. The author repeatedly found the most advanced metamorphosis of the bone-marrow into a uniform, red, gelatinous mass in cases in which the most careful examination of blood preparations showed not a single nucleated red blood-corpuscle.* The diagnosis of pernicious anemia was, however, made by the other blood-findings and necropsy.

An extremely rare occurrence—at least in Germany—is the presence of *flagellata* in the blood of pernicious anemia. According to the concurring statements of Klebs, Frankenhäuser, and Neelsen, however, it would seem that actively motile infusoria—sometimes with, sometimes without, flagella—occasionally appear in the blood. Whether they have any etiologic significance or not is questionable, even though their almost constant occurrence in several cases observed by Klebs in Zürich and Prague points to immediate blood infection.

It has already been stated that the signs of degeneration observed in the blood-findings in pernicious anemia produce a characteristic picture. Whether this degeneration is always primary or whether severe disturbances of the plasma precede the degeneration of the corpuscular elements is still an open question. At all events, the possibility that the process may occur under the latter circumstances cannot, according to the researches of Wooldridge and others, be denied.

The observation of a few cases of pernicious anemia in which a primary osteosarcoma or, contrary to the usual findings, an enormous tumefaction of the spleen preceded the severe general disturbances leads to the presumption that disease of both "blood-forming" organs may give rise to the development of genuine pernicious anemia. But even in these cases, which, because of their extreme rarity, preclude general conclusions, there is lacking the clear insight into the relation of the pathologic processes.

In all anemic subjects the blood-findings are subject to greater variations than in health. As a rule, therefore, a diagnosis should *not* be based upon a single blood examination. This is especially true of the number of leucocytes to which we have, to a certain degree, ascribed an important rôle, in the differentiation of the ordinary secondary and pernicious form. Even in the latter, however, von Noorden occasionally observed an intense leucocytosis of very brief duration, accompanied by improvement in other respects.

Leukemia [Leucocythemia].

In comparison with the blood diseases already considered the diagnosis of developed leukemia is usually more readily and definitely made. Very often a single glance into the microscope suffices to render absolutely certain a presumptive diagnosis based upon other signs. Cases occur, however, in which such a superficial examination is inadequate and which demand most careful microscopic study. In such instances it is necessary not only to count the red and white blood-corpuscles, but it is for many reasons desirable, if not absolutely essential, also to stain and study dried blood-preparations.

The characteristic feature of leukemic blood is a permanent, more or less intense **increase in the number of leucocytes.** It has already been stated that the normal ratio between the red and colorless blood-corpuscles varies between 1 to 500 and 1 to 1000, and a ratio of 1 to 400 observed in frequently repeated

counts made in the intervals of digestion should arouse attention. In leukemia the ratio is so altered that in *many* cases from 8 to 10 or 20 red to 1 colorless corpuscle occur; indeed, a ratio of 1 to 2 and even of 1 to 1 has been described by reliable authorities. Fleischer and Penzoldt found a ratio of even 1.15 white to 1.05 red and Sörensen of 1.70 white to 1.175 red blood-cells.

This affection of the blood was first described by Virchow in 1845 as a separate disease, and first diagnosticated in the living subject by Vogel in 1849. In the majority of instances it attacks the male sex between 30 and 40 years of age. Among the causes are mentioned chronic infectious diseases, such as syphilis and malaria; also chronic enteritis, alcoholism, and especially traumatic influences. The duration of the disease is generally from one to two years; it sometimes runs a very rapid course in from several days to a few weeks. The picture in Plate IX, Fig. 15, was taken from such a case observed by the author in 1892. The patient, 68 years old, said that about three months previous to coming under our care he had suffered from several severe attacks of diarrhea; he continued, however, to perform the arduous duties of a letter-carrier until two and one-half weeks before his death. The case was one of almost pure lymphatic form,—all the lymph-glands showed hyperplasia: the spleen was of normal size and the bone-marrow scarcely altered. A feature of remarkable interest was an extensive *lymphomatous formation in the heart.* In a rapidly fatal case of primary sarcoma of the thymus von Jaksch observed the pathologic features peculiar to lymphatic leukemia, and the author observed the same phenomena in a man, aged 50 years, who died from an enormous carcinoma of the stomach. The author does not believe, however, that it is correct to classify such cases as "leukemia."

Since then a number of similar cases have been described by A. Fränkel and others; the author himself has reported four personal observations in which the intense febrile course suggested the presence of an acute infectious disease; the fifth case was seen by him in October, 1899.

A positive diagnosis of leukemia is possible only by microscopic examination of the blood. The drops of blood withdrawn sometimes appear normal, but oftener pale red, thin meat-juice color, more seldom chocolate colored. In pronounced cases the first glance gives the impression of a considerable increase of the colorless corpuscles. The latter also manifest great variations. More or less decided differences in size and other morphologic deviations are seen. In addition to small and medium-sized leucocytes showing a delicate granulation, many cells with distinct, highly refractive, coarse granulations are generally found.

The red blood-cells are paler than usual, and not rarely of variable size. Blood-plates are usually more numerous than normal. According to numerous authorities, with whom the author agrees, *a little practice will usually suffice* to diagnosticate, by careful examination of the *fresh* blood alone, the particular form of leukemia according as the participation of the spleen, bone-marrow, or lymphatic glandular system *preponderates.* If those colorless corpuscles, which are about as large as a normal erythrocyte, are chiefly increased, it may safely be concluded that the *glandular system* is principally involved (Plate IX, Fig. 16)—"*lymphatic form*"; on the other hand, if the large cells appear in the majority, a participation of the bone-marrow and spleen must be thought of (Plate IX, Figs. 14, 15, and 16) —"*myelogenous and splenic forms*"; and the spleen should be suspected when there appear in every field of the microscope a large number (from 3 to 5 and more) of cells filled with strongly refractive, spheroid granules, while involvement of the bone-marrow is rendered probable especially by the presence of *large mononucleated* leucocytes.

However, an insight into the different features presented by the leucocytes and the ratio of the small, medium-sized, and abnormally large to each other is possible only by means of *stained dried preparations* which also reveal the structure of the nucleated red blood-corpuscles, which are almost *never* absent.

Methods of Staining.—1. Rapid staining with heated 0.5-per-cent. watery or alcoholic solution of eosin and concentrated aqueous solution of methylene blue which is *not* heated. The watery solution of eosin should act for from eight to ten minutes and the alcoholic for three minutes [see page 153].

Similar pictures are obtained by treating for from twenty to thirty minutes with heated Chenzinsky solution. If this staining solution is allowed to act for twenty-four hours in a warm room or near a stove, very instructive results are obtained. Eosinophile and basophile granules are well shown, as are also the nuclei of the red blood-cells. The pictures thus obtained are distinguished from those stained rapidly by the sharper contour of the individual cells.

2. Stain with Ehrlich's tri-acid solution for from *two to six minutes without heating.* According to the author's experience, two minutes' staining barely tinges the eosinophilic granules and the neutrophiles not at all; after four minutes the former are distinctly stained, and the latter just visible; after six minutes a very sharp picture is obtained.

This reveals beautifully the proportion of red to white cells, and also the ratio of the various forms of the latter to each other, as well as the nucleated red blood-cells, the neutrophilic granules of the polynuclear and *large* mononuclear cells, and the eosinophilic granules (Plate IX, Figs. 14 and 15).

3. For demonstration of **mast-cells** staining with concentrated watery solution of methylene blue (for from ten to fifteen minutes) is sufficient.

4. Staining with Ehrlich's eosin and hematoxylin solution for from twenty to twenty-four hours (Plate IX, Fig. 15) gives excellent and highly instructive pictures. The outlines of all the elements are very distinct, and the staining of the various cells and nuclei in different tones of color is such as can scarcely be approached by any other method. The nuclear figures are particularly delicate and well defined, especially the chromatin reticulum in the nuclei of the large mononuclear cells. The staining of the individual blood-cells has already been considered. This method is warmly recommended by the author.

5. Staining for from six to eight hours in 5-per-cent. carbol glycerin saturated with eosin and ammonium picrate stains the red blood-corpuscles yellow and the eosinophilic granules intensely red. Proof is thus furnished that the latter are in no way connected with hemoglobin.

Staining of Fresh Blood Preparations.—It is occasionally desirable to stain the **fresh blood** just taken from the patient. If 2 or 3 drops of a 0.1- to 0.5-per-cent. watery solution of eosin are placed at one edge of the cover-glass and made to flow beneath the latter by absorption with a piece of filter-paper placed at the opposite edge, the red blood-cells will be seen to assume a distinct yellow color; the staining of the eosinophilic granules is especially prominent, and often intense. If some dilute methylene blue solution is now allowed to flow beneath the cover-glass in the same manner, the dye will here and there be quickly taken up by other granules. *On different occasions the author observed several kinds of granulations in one cell in such preparations.* This is emphasized here because Ehrlich denies such an occurrence in one cell (in dry preparations). Rieder has found similar pictures in dry preparations in acute leucocytosis excited by injection of bacterial extracts containing proteid; he designated such cells *"amphophilic"* [ἀμφί = both; φιλεῖν, to love].

Diagnostic Difficulties.—The diagnosis of **developed** leukemia is made quite easily and with certainty. Cases are occasionally met with, however, in which a suspicion of the existence of leukemia is aroused by a series of clinical symptoms, although

examination of fresh and many stained preparations fails to confirm the diagnosis. In such instances repeated careful counts of the blood-corpuscles must be made. To accomplish this generally requires more skill than in staining, aside from the fact that the counting of the white blood-corpuscles is attended by greater errors than is the case with the red cells; if a successfully stained preparation is examined, a quite accurate estimation of the ratio of white to red cells is possible. In this case a great many microscope fields must be counted with the aid of the ocular micrometer — at least twelve hundred. This can very readily be done. In this way the numeric ratio of the *different forms of leucocytes* can also be determined at the same time.

The diagnosis would be greatly simplified if it was possible to recognize a definite type of cell as characteristic of leukemia. Shortly after the discovery of the *eosinophile* cells and the observation of their abundant occurrence in leukemia, it was believed that the appearance of these cells could be accepted as an important diagnostic feature. Years of research have restricted the value of this symptom. It has been shown that eosinophile cells may also occur in large numbers in the blood under many other circumstances: *e.g.*, in asthmatics [and recently in trichinosis]. Likewise, the assumption that the mast-cells are observed only in leukemia has been disproved. On the other hand, Ehrlich considers the large *mononuclear neutrophilic* cells (myelocytes) to be of important significance in the diagnosis of leukemia, especially when associated with the presence of eosinophile cells and nucleated red blood-cells. Long before him, however, Eberth and others drew attention to the striking characteristics of the large mononuclear leucocytes. Even in unstained preparations,— under certain circumstances only after addition of acetic acid,— but much more characteristic in stained preparations, especially in eosin-hematoxylin, the careful observer will see leucocytes which are never or but very rarely present in normal blood. These are *large cells more than twice the size of an ordinary colorless blood-corpuscle* which usually possess but one exceedingly large nucleus, located more frequently eccentrically than centrally, occupying the greater portion of the cell-body, and usually in crescent form. The nucleus is sometimes lobulated or wallet-formed; more rarely polymorphous. The nuclei stained with

hematoxylin show almost throughout a developed reticulum, the chromatin structures of which are distinctly stained, showing the lighter protoplasm in the spaces between. H. F. Müller determined beyond question the occurrence of indirect division in these cells. Since these cells, which also *occur in the normal bone-marrow,* are met with in great numbers in *leukemic bone-marrow,* it is not going too far to regard these large mononuclear cells as "bone-marrow cells" [myelocytes], and to assume that in leukemia they are washed thence into the blood (Mosler, Neumann, and others). (See Plate IX, Fig. 14, *d,* and 15, *c.*) Like the polynuclear leucocytes, the myelocytes have a distinctly granular protoplasm around the nucleus, and there are also three varieties: neutrophile, basophile, and eosinophile myelocytes.

The question arises whether the occurrence of these "marrow-cells" in the blood in doubtful cases may be accepted as absolutely decisive in the diagnosis of leukemia. The author believes that, with certain restrictions, this question may be answered affirmatively. A single conflicting observation must naturally restrict the value of this sign. The author has already referred to a case—that of a very pale woman affected with violent pains in the bones. In this instance almost every field of the microscope showed single "marrow-cells," which now and then presented even faint neutrophilic granulation. There was also decided poikilocytosis. Nevertheless, the rapid and permanent cure following proper care and the use of iron and arsenic forced the conclusion that the case was one of chlorosis gravis combined with anemia.

Therefore, neither the increased occurrence of eosinophilic cells nor the appearance of a moderate number of so-called marrow-cells can be interpreted as *absolutely* decisive in the diagnosis of leukemia. On the other hand, however, *the abundant occurrence of these large mononucleated cells, which seldom, if ever, occur in normal blood,* combined with decided increase in the number of eosinophile cells, is of the *greatest significance for such a diagnosis.* That the marrow-cells [myelocytes] may also contain eosinophilic granules has already been stated; this type appears thus far to have been observed only in leukemic blood. [They are said to occur occasionally in the blood of pernicious anemia.—Brooks.]

Finally, the *motile phenomena* of the leucocytes deserve special consideration. Normally the multinucleated leucocytes are distinguished from the mononucleated by their active motility. The same motility is observed in the ordinary multinucleated eosinophile cells. On the other hand, the "marrow-cells" [myelocytes] manifest no motility upon the warm stage.

A quite rare manifestation in leukemic blood is the presence of isolated *Charcot crystals*. The author has never observed these crystals in the unaltered blood, but he has seen them several times in large numbers in freshly drawn blood after the

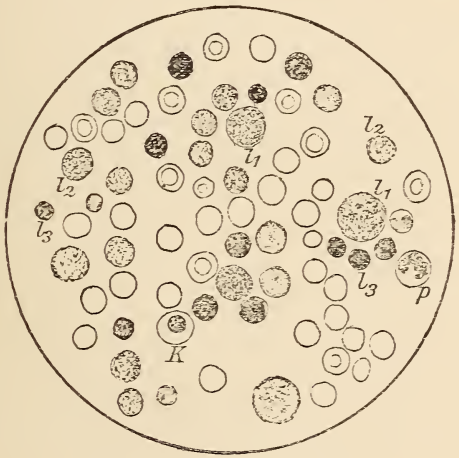


Fig. 84.—Acute Leukemia.

l_1 l_2, l_3, Large medium, and small lymphocytes. p, Polynuclear leucocytes. K, Nucleated red blood-corpuscle.

addition of watery solutions of eosin and methylene blue. This phenomenon has no diagnostic significance. As a rule, they are found in *decomposed* leukemic blood, though they may appear in the absence of such a change.

In so-called **acute leukemia** large numbers of *mononucleated* leucocytes are chiefly met with. After *six* personal observations of this kind it appears to the author to be wrong to consider any special feature as characteristic. In Plate IX, Fig. 16, are given the findings in two cases which were clinically very similar; in one the small mononuclear, in the other the medium-sized and large mononuclear cells chiefly are increased. It is important to

note that in all cases the multinucleated leucocytes are diminished in number. The author would lay emphasis upon the fact that in his last observation the lymphocytes almost exclusively were increased, although *enormous tumefaction* of the *liver* and *spleen* existed in addition to the intense general swelling of the lymphatic glands (see Fig. 84).

Whether the pathologic condition designated as acute leukemia should be regarded as an acute form of this disease appears to the author very questionable.

The causes of the alterations in the blood in leukemia have not yet been explained. The question whether the process is an independent disease of the blood, with retarded disintegration of the more resistant leucocytes (Löwit), or whether the blood-forming organs themselves have become permeable and are no longer capable of preventing the premature escape of unripe corpuscular elements (Virchow), is still unsolved. Perhaps both views are correct, and the process is one in which there is hyperplasia and abnormal permeability of the blood-forming organs as well as a diminished destruction of leucocytes in the circulating blood. It is certain, however, that a very active formation of cells by division can be observed in those locations in which blood-formation occurs (Bizzozero)—a fact which militates against Löwit's theory. Likewise, in almost every case of leukemia there is to be found a more or less advanced hyperplasia of the three blood-forming organs: the spleen, the lymphatic glands, and the bone-marrow. The latter is greatly altered, particularly in the long tubular bones and sternum, but also in the ribs and vertebræ. According to Neumann, to whom we are chiefly indebted for our knowledge of the subject, the marrow appears either puslike (pale greenish) or of a more homogeneous raspberry-red color and of pyoid or lymphadenoid form. As a rule, the varieties of leukemia met with are mixed types in which all blood-forming organs present alterations, the intensity of the pathologic process varying in different localities, one place showing less advanced, another intensely progressive changes. The question as to which organ is *most* affected can generally be decided by the blood-findings.

The case of Leube and Fleischer, which at necropsy showed lymphadenoid changes in the bone-marrow, but no alterations in the spleen and glands, is usually quoted in support of Löwit's theory. This observation, however, by no means offers a firm basis for his theory; for it does not explain why in this case a *deposition* of the leucocytes accumulated in the blood did not occur in the spleen and glands—a phenomenon which, according to Löwit, always occurs *secondarily* in leukemia. That medullary alterations may be entirely absent even in leukemia of intense degree has been demonstrated by Fleischer and Penzoldt.

Leucocytosis.

In connection with leukemia we will briefly consider those abnormal states which may give rise to confusion with it. Aside from certain gross, palpable phenomena resembling leukemia, which will later be discussed, a transitory increase of leucocytes especially arouses suspicion. The number and kind of these cells must, then, decide the question.

In *leucocytosis* there is observed a more or less decided, usually *transitory, increase of the colorless corpuscles normally present in the blood.* The increase is seldom of high degree, although von Jaksch has described in children a leucocytosis with a ratio of 1 to 12. [The leucocytes seldom run higher than 30,000 per cubic millimeter, though in rare instances the number may exceed 100,000.—Brooks.]

The conditions under which leucocytosis may occur are partly of a physiologic, partly of a pathologic, nature.

1. Physiologic Form.

During the *period of digestion* there generally occurs a distinct increase in the number of colorless elements. According to the unanimous opinion of numerous investigators, however, this phenomenon is neither invariably observed nor always of the same degree of intensity in the same individual.

In healthy subjects the increase in the number of leucocytes usually begins shortly after eating. After from three to four hours it attains its maximum, which exceeds by 3000 the number (8000) usually contained in 1 cubic millimeter of blood. The ratio of mononuclear and polynuclear cells generally remains unaltered.

Digestion leucocytosis is more pronounced in healthy individuals than in diseased subjects, especially such as suffer from digestive disturbances. In *gastric cancer* especially physiologic leucocytosis has frequently been found to be absent, while it is said to be constantly observed in gastric ulcer. Further research and observation in this direction are highly to be desired. There appears to be little doubt but that digestion leucocytosis attains a much greater degree of intensity in children than in adults, and that, to a certain extent, it runs parallel with the proteid supply.

This fact may, perhaps, offer an explanation for the occurrence of digestion leucocytosis; it is quite possible that the leucocytes are stimulated by the products of proteid metabolism, especially by peptone.

Through experiments made by Pohl upon dogs deprived of food for eighteen hours in order to avoid error from gradual absorption of food still present in the intestine, it was found that, as a rule, a distinct leucocytosis occurred only after the administration of *albuminoid* substances. It began one hour after feeding and reached the maximum in three hours at the latest.

Physiologic leucocytosis is also observed during *pregnancy*, especially in the second half of the period of gestation. Primipara manifest it regularly, while multipara offer exceptions. Long ago Virchow demonstrated a growing increase of the leucocytes in pregnant women from month to month, and attributed the phenomenon to progressive expansion of the uterine lymph-vessels, and more active metabolism and hyperplasia of the inguinal and lumbar lymphatic glands. Careful counts made by Rieder upon thirty-one gravida, who had fasted for from fourteen to sixteen hours, showed decided variations in the leucocytes, in twenty ranging from 10,000 to 16,000, and an average increase of the number to about 13,000 per cubic millimeter. About one-third of all the leucocytes belonged to the mononuclear forms.

Finally, constant and often pronounced leucocytosis occurs in the *newborn*. According to Hayem, Rieder, and others, the number of colorless cells exceeds that normally present in adults from twofold to threefold. The highest numbers occur in the first three or four days after birth; a diminution then occurs which sometimes continues until the number approaches that observed in the adult. Usually, however, a rapid rise soon reappears, and even in the third and fourth weeks the number reaches an amount 50 per cent. in excess of normal. The increase affects the mononuclear as well as the polynuclear forms—usually the former and especially the small mononuclear. A perceptible increase of the eosinophile cells is also generally present.

Furthermore, there is observed in the newborn an augmentation in the number of erythrocytes, *some of which are still*

nucleated, and a more or less decided increase in the amount of hemoglobin (about from 25 to 30 per cent.). In addition, poikilocytes and microcytes are not infrequently met with.

A sufficient explanation for this very remarkable deviation is still lacking. The fact that the number of red and white blood-cells and the amount of hemoglobin are considerably increased renders it probable that, in the newborn, there is a general overproduction intended to augment the powers of resistance of the young being just liberated from the maternal organism, and to serve as a reserve fund which is made necessary, perhaps, by the suddenly altered conditions of life and the constant and relatively significant loss of weight.

2. Pathologic Form.

Pathologic leucocytosis is of *regular* occurrence in *chronic* cachectic disturbances, after severe loss of blood, and shortly before death [agonal leucocytosis].

In the first-mentioned cases the blood presents more or less distinctly the features of *hydremia;* the latter appears more readily after loss of blood *when a number of small, rather than when single large, hemorrhages have occurred.* With the occurrence of every hydremia there is an increased lymph-supply (Cohnheim, Lichtheim), which leads to a rise in the number of leucocytes in the blood. Swelling of the lymphatic glands plays an insignificant, if any, rôle. Chronic hydremic leucocytosis may vary within wide limits. Increases amounting to from 20,000 to 30,000 per cubic millimeter have been observed. The part the different forms of leucocytes play in the increase varies. Sometimes the mononuclear, sometimes the polynuclear are decidedly increased. *The latter occurrence appears to be the rule in carcinomatous cachexia.*

The cause of **terminal leucocytosis** [leucocytosis of the moribund] is not clear. Possibly the fall in the blood-pressure, perhaps also the action of certain toxic products, play a rôle in its production. At any rate, its occurrence is unquestionable; and to this source also may be attributed the increase in the number of leucocytes noticed toward the end of life in many cases of pernicious anemia, in which a leucopenia usually exists.

The occurrence of an often enormous increase in the number of leucocytes in a series of acute diseases, especially of an infectious nature, is not quite so uniform as under the above-

mentioned conditions. This form is designated as **inflammatory leucocytosis,** the nature of which has been critically studied only within the last decade. Our knowledge of the subject has, however, been so far advanced that the occurrence or absence of inflammatory leucocytosis is now accepted as a differential diagnostic and prognostic sign. At the present time the following may, in a measure, be accepted as proved:—

The highest degree of inflammatory leucocytosis is observed in **acute lobar pneumonia;** the increase occurs here within a few hours after the chill, rapidly reaches the height of from 20,000 to 30,000, or even 60,000, cells per cubic millimeter, falls very decidedly after twenty-four hours, but remains more or less above the normal until the crisis, and falls still farther only with the decline of temperature, again reaching the normal number a few days later. [Before the crisis the polynuclears constitute 80 per cent. of the total number of leucocytes. On the eve of the crisis they rise to 80 per cent., falling to 71 per cent. after defervescence, and to 57 per cent. on the days following (Leredde). At this time there is observed an increase in the number of lymphocytes and eosinophiles, the latter varying from 3 to 5 or even 7 per cent. (Turck). In cases in which polynucleosis persists after defervescence, the termination of the disease should, according to Roger, be considered incomplete; if the polynuclears increase, suppuration of the exudate should be feared.—Brooks.]

In cases ending fatally the increase, if present at all, is insignificant. A definite parallelism between the degree of leucocytosis and the local and general pathologic phenomena has not yet been positively determined. The increase affects to a preponderating degree the *multi*nucleated forms; and while the eosinophile cells are usually absent, the lymphocytes may be even relatively diminished. A post-apyrexial reappearance of leucocytosis, accompanied by slight rises of temperature, is often the first sign of a beginning empyema.

In contrast to these remarkable results, the researches of numerous authors (Halla, von Limbeck, von Jaksch, and others) have made it probable that in *typhoid fever* an inflammatory leucocytosis is not only *absent,* but a diminution of the leucocytes (to from 5000 to 1800 per cubic millimeter) [leucopenia] is the

rule. The reduction affects especially the multinucleated cells; it occurs in all stages of typhoid fever and disappears only on recovery.

An explanation of this phenomenon is difficult in view of the fact that Buchner designates the protein of the typhoid bacillus as strongly chemotactic. [While in the beginning of typhoid there is a reduction in the number of leucocytes (Millet, Stienon, Chantemesse), the polynuclears are more numerous than normal,—70 to 90 per cent.,—the lymphocytes are diminished, and the eosinophiles almost entirely absent. The number of leucocytes is always subnormal in the second stage; the number of mononuclears, particularly the lymphocytes, rises while the number of polynuclears declines by sudden falls. During convalescence the polynuclears are not markedly increased, the lymphocytes are increased, and the large mononuclears constitute 20 to 30 per cent. of the total number of leucocytes (Roger). The approach of convalescence is said to be announced by an increase in the number of eosinophiles. A sudden augmentation in the number of polynuclear leucocytes is indicative of some inflammatory complication (pneumonia, etc.).—Brooks.]

In addition to typhoid, inflammatory leucocytosis is *absent* in *measles* and *tubercular meningitis*. Contradictory statements are made in connection with *scarlatina*, von Limbeck and Pick claiming never to have seen leucocytosis, while Rieder found it to be almost constant. [According to Roger, scarlatina is attended by a leucocytosis which persists long after the fall of the temperature. The polynuclears are slightly decreased at the beginning and become more numerous as the disease progresses. Kotschetkoff states that a leucocyte-count of from 10,000 to 20,000 is found in mild cases, 20,000 to 30,000 in cases of moderate severity, and above 30,000 in grave cases. The polynuclears constitute 85 to 98 per cent. of the total number. In average cases of moderate severity the eosinophiles progressively increase up to the second or third week (maximum, 8 to 16 per cent.), and thus gradually return to normal.—Brooks.] In peritonitis, pleuritis, and tubercular meningitis the number of leucocytes is usually found to be within physiologic limits or rather diminished, while in *primary meningitis* an *intense* leucocytosis always occurs.

More or less decided increase of the leucocytes is also observed in sepsis, puerperal fever, erysipelas, *acute* articular rheumatism, diphtheria, recurrent fever, and osteomyelitis. [According to Chantemesse and Rey, in erysipelas in adults who recover the polynuclears undergo a diminution until recovery is established. During the febrile period the

mononuclears do not manifest much variation, but they are considerably increased immediately before or at the beginning of defervescence. The variations of the leucocytes follow a reverse course, and an increase in their number is said to be a certain sign of cure. The eosinophiles are usually absent during the febrile period, but reappear at the time of defervescence. In fatal cases hyperleucocytosis always exceeds 12,000 and is characterized by a polynucleosis reaching or exceeding 92 per cent. (Roger). The persistence or sudden return of polynucleosis during convalescence indicates imminence of a relapse.—Brooks.] Furthermore, many authors have called attention to a pronounced leucocytosis and particularly a decided increase of the eosinophile cells *after the injection of tuberculin* (Botkin). [According to Hayem, in chronic tuberculosis the leucocyte-count reaches from 10,000 to 20,000. When a caseous focus breaks down and purulent expectoration (cavity) occurs, the leucocytes may occasionally run as high as 36,000 (Roger). Aside from whooping-cough, the etiology of which is unknown, *mononucleosis* is usually observed in protozoan infections. During a paroxysm of *malarial fever* an increase in the number of lymphocytes first occurs and a lesser augmentation in the number of eosinophiles and large mononuclears; fifteen to twenty minutes later the number of lymphocytes increases and large mononuclears are rare. There is scarcely any change in the number of polynuclears. Mononucleosis is also observed in *variola*. The number of leucocytes soon varies from 10,000 to 35,000. Although leucocytosis is present at the outset, it is particularly marked at the time of vesiculation and remains stationary, slightly diminished, or increased during pustulation. In the hemorrhagic form it is less pronounced, but is seldom absent (Roger). In *tetanus* leucocytosis varies from 15,000 to 20,000 per cubic millimeter.—Brooks.]

A point of diagnostic importance is the fact that the results thus far obtained may be advantageously employed in those not infrequent instances in which it is desirable to make a differential diagnosis between croupous pneumonia and typhoid fever, on the one hand, and purulent and tubercular meningitis, on the other. In these instances a normal or subnormal leucocyte-count will decide the diagnosis in favor of typhoid fever or a tubercular process as against croupous pneumonia and purulent meningitis. Curschmann has recently called attention to the fact that the demonstration of a leucocytosis in perityphlitis [appendicitis] is of the highest diagnostic significance. If the number of leucocytes rises above 22,000 during the course of this affection, this is an almost unfailing sign of abscess-formation. Immediately after spontaneous or operative emptying of the abscess the leucocytosis disappears. After numerous investigations in this con-

nection carried out in my clinic I can fully confirm Curschmann's statements.

In order to obtain an insight into the nature of inflammatory leucocytosis experimentation has been resorted to. Von Limbeck saw intense leucocytosis after injection of cultures of bacteria, especially those of staphylococcus; Binz and Meyer mention the occurrence of considerable increase in the leucocytes after the administration of ethereal oils; Pohl after spices, etc. The observations of Buchner and others render it probable that it is not the *toxic* (decomposition) products of the bacteria, but chiefly or exclusively their *proteins* (albumin substances) which, according to Pfeiffer's method, produce so-called *positive chemotaxis*—that is, an attraction of the leucocytes. The fact that after extirpation of the spleen, as well as in the above experiments, nucleated red blood-cells and a considerable leucocytosis are observed, speaks somewhat in favor of the view that an irritative action is exerted upon the "blood-forming" organs. Be this as it may, the increase of leucocytes in the blood could also be produced by the entrance of wandering cells or as the result of rapid cell-division within the blood-channels (Löwit). The first hypothesis would be contrary to Ehrlich's theory, since, according to him, only mononucleated cells are supplied to the blood; the great majority of the colorless cells found in acute leucocytosis are unquestionably of the polynuclear variety. However, we know that the transformation of mononuclear into polynuclear cells takes place quite rapidly.

The fact that the termination of those diseases in which inflammatory leucocytosis occurs at all is favorable when the leucocytes are considerably increased and unfavorable when they are diminished suggests that this process is a salutary one. A satisfactory explanation of acute inflammatory leucocytosis is still lacking.

The blood of leucocytosis, when observed upon the warm object-stage, reveals *active motility* of the leucocytes, which are chiefly of the polynuclear type. On the other hand, large mononuclear granulated cells, the occurrence of which in great numbers is, in a measure, characteristic of leukemia, are distinguished by almost complete absence of ameboid movements.

Pseudoleukemia.

The gross, palpable changes which are to be observed in this disease not infrequently present a striking resemblance to the features of genuine leukemia. It is here especially that microscopic examination of the blood is very often of decided diagnostic significance. Although there is often considerable

hyperplasia of numerous, noncaseating lymph-glands and not rarely a considerable enlargement of the spleen and tenderness of the bones, microscopic examination of the blood presents at first either no deviation from the normal or, later, a reduction of the erythrocytes to from 1,500,000 to 2,000,000, according to the degree of anemia, with but only a *slight* increase of the colorless elements. The hemoglobin content is reduced in proportion to the reduction of the number of blood-corpuscles. The change corresponds, therefore, to the signs present in *secondary* anemia.

Now and then swelling of the glands and spleen is observed in patients in whom examination of the blood leaves a *doubt* whether leukemia or pseudoleukemia is present. At first glance a considerable increase of the colorless cells is noticed. An accurate count, however, shows, for example, a ratio of only 1 to 160; if, in addition, nucleated red cells and cells resembling myelocytes are seen in stained preparations, differentiation is difficult or even impossible.

Pseudoleukemia occurs from two to three times more frequently in men than in women and may appear at any age. The possibility of its development into true leukemia, as many authorities assert, has not been positively determined. The very appropriate designation "pseudoleukemia" was first applied to the symptom-complex (first described by Hodgkin) by Wunderlich.

Hemoglobinemia.

In this disease, which has been accurately studied only during the last decade, highly characteristic changes appear in the blood which indicate a more or less considerable destruction of red blood-corpuscles. It is observed after poisoning with chlorids [nitrites], naphthol, pyrogallic acid, hydrochloric acid, arseniureted hydrogen, sulphonal, phenacetin, antifebrin [acetanilid], antipyrin, *fresh* mushrooms, or in connection with acute and chronic infectious diseases (scarlatina, typhoid fever, malaria, and syphilis); also after prolonged exposure to high degrees of heat and cold and after transfusion of animal blood into man; and, finally, it may occur spontaneously as the so-called *paroxysmal*, or *intermittent*, form. Especially predisposed persons are attacked by the disease after violent muscular exertion (only after prolonged marches) or on sudden exposure to cold. It begins with chill and great prostration, and leads rapidly to apparently grave constitutional disturbances and pronounced *hemoglobinuria* (see page 303). In the majority of instances, however, it ends in rapid recovery, until after a time a new attack is produced by similar causes.

If the blood obtained from such a patient by means of a wet cup—or better by means of a syringe from a vein of the arm—is allowed to remain quietly in a test-tube for from twenty to twenty-four hours,—preferably in the ice-chest,—the serum will show a distinct *ruby-red* instead of the ordinary light-yellow color.

In persons suffering from "paroxysmal hemoglobinuria" a purely local alteration of the blood can be produced. If the finger of such a patient is constricted and immersed for fifteen minutes in iced and then in lukewarm water, the serum after separation will show the ruby-red shade even in a thin capillary layer (Ehrlich).

There can be no doubt that this reddening of the serum is caused by the hemoglobin derived from the red blood-corpuscles.

Microscopically there will be found in the freshly drawn blood (also in that from the constricted finger) a slight tendency of the erythrocytes to the formation of rouleaux, a distinct poikilocytosis, and more or less numerous, very pale or entirely decolored disks: the so-called (Ponfick's) *shadows*. The latter deserve especial attention, because they do not occur in any other blood affection. They are, therefore, to be interpreted as a positive sign of hemoglobinemic change.

According to Ponfick's researches, poikilocytosis may be entirely absent, only these shadow forms being present, the hemoglobin having been extracted from the unaltered blood-disks.

In most clinical cases the change in the blood is so pronounced that the spleen and liver, which ordinarily serve to take up disintegration products, become insufficient, and the excess is conveyed to the kidneys. In the liver the hemoglobin is converted into biliary coloring matter, which may appear in abnormally large quantity in the urine, unaccompanied by hemoglobin. If the solution of the hemoglobin is very considerable, the urine contains blood-corpuscle *detritus* as well as biliary coloring matter; hemoglobinuria is produced.

Viewed through the *spectroscope,* the separated ruby-red blood-serum shows distinct O-Hb absorption bands, which establish the diagnosis.

In not rare instances of hemoglobinemia, particularly in those which follow poisoning with chlorids and other substances,

the progressive solution of blood-corpuscles induces pronounced *methemoglobinemia.* Methemoglobin, which has been accurately studied by Hoppe-Seyler, Kuelz, and Huefner, is a union of oxygen with the blood-coloring matter [hemoglobin], in which the same amount of oxygen is present, but in much closer combination. This substance is especially characterized by a strong absorption band in the center of the *red* portion of the spectrum (Fig. 70), in addition to which both O-hemoglobin bands may still be preserved. That portion of the spectrum to the right of the band present in green is usually completely obscured. The band is found in fresh blood, and in that diluted with water, as well as in the urine and in the serum separated in the cold. Its occurrence can be recognized even with the naked eye by the *sepia* or *chocolate color of the blood.*

In 1887 the author spectroscopically demonstrated methemoglobinemia even in the *circulating* blood of the aural vessels of animals (dogs) poisoned with hydrochloric acid salts. It is quite probable that in pronounced cases the same observation can also be made in the human ear. Aside from the immediate diagnostic value, such a determination is of decided scientific interest, since Stokvis states that the development of methemoglobin is possible only *external* to the circulating blood—an assumption which has been disproved by Marchand upon the basis of a large series of complicated experiments, and which can be rapidly and positively proved erroneous by spectroscopy of the circulating blood of the ear.

For clinical purposes the methemoglobin spectrum can easily and quickly be demonstrated by adding a piece of potassium ferricyanid to a fresh solution of blood. Usually an absorption band at once appears in the red of the spectrum, while the O-Hb bands become indistinct or disappear entirely.

Carbon Monoxid Poisoning.

In the diagnosis of this form of poisoning the spectroscope plays a much more important rôle. In such cases oxygen is displaced from its combination with hemoglobin and CO-hemoglobin produced, which is incapable of taking up oxygen. The arterial as well as the venous blood then assumes a *bright cherry-red* color.

Microscopically there are *no* unequivocal *changes* in the blood; the *spectroscope,* however, reveals characteristic phenomena which present *absolute proof of CO poisoning.* While both

absorption bands of oxyhemoglobin lying between the lines *D* and *E* at once disappear on addition of a dilute solution of yellow ammonium sulphid, and are replaced by a single band indicative of deoxidized, reduced hemoglobin, both absorption bands visible in CO poisoning, which also lie in yellow and green, but somewhat nearer each other, are unaltered by addition of ammonium hydrosulphid (see Fig. 70, *a* and *b*). In all spectroscopic examinations it is advisable to compare the blood to be examined with control specimens. I, therefore, mention the fact that CO blood can at any time be prepared by allowing some illuminating gas to flow into normal blood-solution through a bent glass tube.

CO poisoning is most frequently caused by gases liberated from improperly constructed heating apparatus and furnaces, in the manufacture of coke, and also by illuminating gas. Dyspnea, sopor, and convulsions are the main symptoms, which are chiefly referable to the asphyxia resulting from lack of oxygen.

Micro-organisms in the Blood.

As regards bacteria and animal parasites occurring in the circulating blood, all that is of importance will be found in Section I. Here reference will be made only to the fact that thus far the following micro-organisms have been demonstrated in human blood: The spirilla of recurrent fever, the pus-cocci and pneumococci; Friedländer's diplobacillus pneumoniæ, E. Fränkel's gas bacillus and other anaërobic bacilli (personal observation in puerperal fever), tetragenus, proteus (personal observation), diphtheria bacilli, meningococcus intracellularis (Weichselbaum) (personal observation); tubercle, leprosy, glanders, anthrax, typhoid, paratyphoid, and colon bacilli, and also malarial plasmodia and the embryos of filaria sanguinis.

Rare Blood-findings.

In **lipemia** small droplets of fat have occasionally been seen. These droplets have been positively proved to be fat by their staining with osmic acid, their strong refractive index, etc.

In **melanemia**, which is seen only as a result of pernicious malaria, small granules and large flakes of pigment may be noticed in the blood either during or long after the attack.

THE FORENSIC IDENTIFICATION OF BLOOD-STAINS.

For forensic purposes it is sometimes necessary to make an examination to determine whether certain red stains found upon clothing, floors, walls, etc., are caused by blood, and especially human blood. In most instances this question can be decided by microscopic demonstration of still remaining blood-corpuscles or their coloring matter.

As a rule, red blood-corpuscles can be recognized only in comparatively fresh blood-stains. Fresh blood adhering to any of the above-mentioned articles can be identified by simply moistening small particles which have been carefully scraped off, or the blood-soaked fibers, etc., in a physiologic salt solution and watching developments under the microscope. When the stains are older, it is necessary to macerate the blood-traces for several hours in 30-per-cent. caustic potash solution or in Hofmann's modification of Pacini's fluid. This consists of:—

Corrosive sublimate	1 part.
Common salt	2 parts.
Water,	
Glycerin .....of each	100 parts.

In the case of old, dried blood-stains, small particles may be placed in a watch-glass and subjected for several hours to the action of the above reagents, or a small quantity may be scraped off with a needle and placed upon a glass object-slide, then 1 or 2 drops of the above solutions added, and the gradual separation of the coherent blood-disks watched under the microscope. The observation of the progressive development of a number of rounded disks out of the originally amorphous mass is characteristic. As a rule, a decision can be made with the microscope only in so far as to whether the red blood-cells observed are human or mammalian, because these are always nonnucleated, while those of the other vertebrates (birds, fish, frogs) are nucleated. On the other hand, the question of the presence of human blood may remain undecided, owing to the great similarity in form of the red blood-cells of man to those of certain domestic animals.

A decision upon the basis of comparative measurements of the red blood-corpuscles can be made only in fresh blood, since the red blood-cells of man (7.9 μ) are larger than those of mammals, of which the dog (7 to 7.4 μ), ox (6 μ), and the horse (5.8 μ) have the largest erythrocytes. In the case of old blood-stains, maceration in 30-per-cent. caustic potash solution will be most likely to render it possible to measure the diameter of the separated cells. Now and then, even after years of drying, decided differences in size are still quite distinctly recognizable; for example, the difference in the diameter of human

blood-cells and those of the sheep (4.5 μ). In such cases, however, it is absolutely essential to measure a *large* number of corpuscles, because notable differences in size occur even in normal blood.

From the foregoing it is plain how difficult it is in the microscopic examination of questionable blood to identify the elements as from the human subject. It is therefore gratifying that a biologic method has recently been found which, even after years, enables us to determine the origin of traces of blood: *i.e.*, to differentiate human from animal blood. The principle of the method is based upon the fact that a clouding and precipitation (**precipitin reaction**) occurs in a blood-solution when the latter is mixed with homologous serum: *i.e.*, with serum obtained from an animal which has previously been treated with the same kind of blood. As the procedure requires special technical skill, we must refer, for the minutiæ of the test, to the original literature on the subject.

Rindfleisch's admonition not to mistake the spores of some of the lower fungi (achorion Schönleinii) for small blood-cells is worthy of the most careful consideration. Aside from certain microscopic characteristics recognizable by the practiced eye, these spores are distinguished from blood-cells by their greater resistance to the action of acids and alkalies.

In almost every case it is advisable also to employ other methods of identification.

The presence of blood-coloring matter is demonstrated microscopically by the production of the **hemin crystals** discovered by Teichmann. The presence of these depends upon the fact that hematin, even in minute blood-stains, combines with hydrochloric acid to form very characteristic crystals. Teichmann's method is carried out as follows:—

Place a small drop of physiologic salt solution upon a glass object-slide and evaporate by aid of gentle heat. Upon the delicate crystalline layer thus formed place a small portion of the dried and finely powdered blood-scrapings or minute fragments of the stained fabric, and cover with a cover-glass [interposing a hair]. Now add enough *glacial* acetic acid completely to fill the space between the cover-glass and slide, and heat for from three-fourths to one minute over a [Bunsen or alcohol] flame, until *minute* bubbles appear. Renew the acid, 1 drop at a time, as it evaporates, until a delicate red-brown color develops. As soon as this is noted, the remaining acetic acid is allowed wholly to evaporate at some distance above the flame. Allow glycerin to flow in from the edge of the cover-glass, to clear and preserve the specimen. [And then examine with $^1/_5$ or $^1/_6$ lens, when the delicate, characteristic, brownish crystals of hemin will be seen, provided the reaction has taken place. It is advisable to repeat the test if the first trial is unsuccessful. Care should be taken not to heat the specimen too strongly.—Brooks.]

Even with the unaided eye, if blood is present, there will be seen here and there under the cover-glass bloody or brownish-red dots and streaks. After these have been located with the low-power objective [$^2/_3$], their finer details are examined with a magnification of 250 to 400 (see Plate IX, Fig. 17).

Hemin or hydrochlorate of hematin crystals are light- or dark- brown, rhombic plates or columns of very variable length and breadth. Occasionally whetstonelike forms of the same color are also met with. The size of the crystals depends to some extent upon the method of preparation: the more slowly and carefully the acid is allowed to evaporate, the more frequently large pieces 15 to 18 μ in length will be found. In every instance, however, the greatest variety in size can be seen, from those barely visible up to the measurements just mentioned. They occur singly or in masses, and often assume the form of St. Andrew's cross; very often they lie crossed upon each other. They are insoluble in water, alcohol, and ether, are readily soluble in caustic potash, and are soluble with difficulty in acids and ammonia.

Their demonstration is not attainable in every instance; aside from the fact that, in the absence of sufficient care the cover-glass is liable to crack, their formation may be hindered or rendered impossible by the presence of fat, rust, or advanced changes in the blood-coloring matter. If fat is the disturbing substance, it must be extracted with ether.

The spectroscopic demonstration of blood-coloring matter is of as great value in the diagnosis of old blood-stains as is the detection of hemin crystals. As has frequently been stated, the two absorption bands lying between the lines *D* and *E* of the solar spectrum make it at all times possible to recognize a solution of oxyhemoglobin. Both bands appear distinctly even when the tinging of the solution is scarcely perceptible, and they positively prove the presence of hemoglobin. To guard against error, which is possible only with the similar spectrum of *ammonium carminate,* the following two tests may be employed: 1. On addition of yellow ammonium sulphid, the two absorption bands of oxyhemoglobin disappear and a single broad absorption band peculiar to reduced hemoglobin takes their place; on shaking the solution with oxygenated air the single band disappears, and

the two absorption bands of oxyhemoglobin are restored. 2. On addition of a little acetic acid both absorption bands of oxyhemoglobin disappear, while the similar bands of ammonium carminate persist unaltered. If both of these tests result positively, incontrovertible proof that the suspected stain contained hemoglobin is obtained [see Fig. 70].

The necessary prerequisites for securing the above-described spectrum-findings are solubility of the blood-stain in water, and a quantity sufficient to yield a watery solution from one and one-half to two centimeters high in an ordinary test-tube. The slit of a pocket spectroscope is now placed against the test-tube, and the strongest possible light allowed to pass through the solution.

If only minute traces of blood are present, the examination should be made with the microspectroscope, which is inserted into the draw-tube in place of the ocular. The blood-stain must then be dissolved in a diminutive glass chamber having plano-parallel walls, and if the solution is turbid it should be cleared by the addition of a trace of ammonia (by which the globulin is dissolved).

The spectrum of methemoglobin, which, in addition to the bands of oxyhemoglobin, also shows one in *red,* has already been discussed (page 130). It is to be especially borne in mind during medico-legal investigations that methemoglobin belongs to the frequent metamorphosis phenomena of oxyhemoglobin, which occur as the result of the action of sunlight and air. On addition of a small amount of yellow ammonium sulphid or ammonia the characteristic band in red disappears; on the other hand, yellow ammonium sulphid produces the broad band of reduced hemoglobin instead of the two bands of oxyhemoglobin; while on addition of ammonia both bands not only persist, but are made more distinct.

In the case of very old blood-remains which have been decomposed by the action of light and air and which are *insoluble in water,* spectroscopic demonstration of **reduced hematin** may still lead to the desired result.

For this purpose the suspected blood-stain is treated with a from 10- to 20-per-cent. solution of potassic or sodic hydrate (Stokes) or saturated solution of potassium cyanid (E. Hofmann) until dissolved, and the solution thus obtained (either undiluted or diluted with water) placed in front of the slit of the spectroscope. If reduced hematin is present, there appears an absorption band resembling that of reduced

hemoglobin, except that it is displaced toward the yellow of the spectrum; its specific nature is shown on addition of yellow ammonium sulphid; while, as we are aware, the two oxyhemoglobin absorption bands blend into one broad band, here—on the contrary—the single broad band separates into two parts, which are especially characterized by their situation in the green of the spectrum.

III. EXAMINATION OF THE SPUTUM.

Affections of the respiratory organs are usually accompanied by expectoration. Under expectoration is included all secretion discharged through the mouth by hawking and especially by coughing. Between expectoration and coughing there exists, as a rule, an immediate relative dependence in so far as severe coughing usually promotes profuse, and less frequent and mild coughing slight, expectoration. There are, however, many exceptions to this rule.

Coughing is often very intense, still "nothing is loosened," because little, or only very tough, secretion is present; or expectoration is slight even in violent coughing, because the patients at once swallow the greater portion. The latter is the rule with children (up to the sixth or seventh year), and often the case in weak elderly or seriously ill (typhoid, pneumonia, delirious, etc.) patients. On the other hand, large amounts of sputum are not infrequently raised by slight coughing or even by simple contractile movements of the chest (bronchoblennorrhea, bronchiectasis).

The sputum is usually of great semeiotic significance, for it may furnish information in regard to the pathologic processes occurring within the respiratory apparatus. It is clear, however, that in addition to *essential* constituents the sputum will also contain a number of unimportant elements added to it during transit through the air-passages. To the former may be referred such constituents belonging to the anatomic structures and which may be thrown off in inflammatory and necrotic processes; also such as occur only in disease as etiologic factors or sequelæ of an especial disease. To the second group belong such elements which, for example, gain entrance to the sputum from the oral cavity or are added to it outside the body through the agency of unclean receptacles—spit-cups, etc.

For the proper judgment of the *essential constituents of the sputum* an accurate knowledge of the anatomic structure of the respiratory tract is absolutely necessary. For this reason a short histologic sketch will first be given,

The nasal mucous membrane in the movable part of the nose is covered with lamellated squamous epithelium, and in the pars respiratoria with ciliated columnar epithelium. Likewise the mucous membrane of the larynx, trachea, and larger bronchi is covered by stratified ciliated epithelium, which is interrupted by mucus-secreting goblet-cells. Only the posterior surface of the epiglottis, the anterior surface of the arytenoid cartilage, and the true vocal cords are covered with lamellated squamous epithelium.

The epithelium of the bronchial mucous membrane (Fig. 85) gradually loses lamellæ until in the finer branches only a single layer of ciliated epithelium is present, which is continued to the beginning of the bronchioles. The ciliated epithelium, however, gradually passes over into an epithelium composed of a mixture of cubic and large nucleated and nonnucleated cells, which even in the neighborhood of the alveolar ducts consist chiefly of the large *polygonal, flat,* so-called *respiratory epithelium.* It has been found by embryologic research that the epithelium

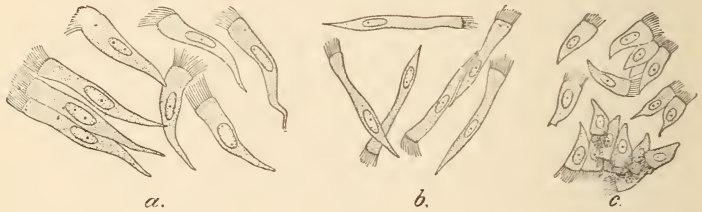


Fig. 85.—Ciliated Epithelium (Obtained by Careful Scraping of the Mucosa). × 350.

a, From a principal bronchus. *b,* From a fine bronchus. *c,* From a bronchiole.

gradually becomes *flattened* only after respiration has become established. In stillborn children cubic epithelium only is found in the alveoli.

Smooth muscle-fibers accompany the bronchial tube down to the alveolar ducts and *form a delicate ring at the point of origin of the alveoli.* Besides these muscle-fibers the *wall of the alveolar ducts is rich in elastic fibers, which are arranged as circular fibers and also surround the opening of each alveolus and from there send out branches which support the whole alveolus. By the immediate continuation of neighboring elastic-fiber rings the alveolar septa are formed.* The respiratory portions of the lungs are divided by connective tissue into small and minutest lobules; in the intertubular fibrous stroma are found black pigment and minute carbon granules which have been deposited there by respiration and the lymph-current.

In spite of the achievements of physical diagnosis, the **essential parts of the expectoration** often first decide the diagnosis. Sometimes the characteristic feature is recognizable with

the naked eye, sometimes only by aid of the microscope. For example, a stinking sputum mixed with tissue-threads often at once reveals the existence of pulmonary gangrene, while physical phenomena on the part of the lungs may, perhaps, be but slightly developed; on the other hand, microscopic examination of a stained preparation of the sputum may assure a diagnosis of pulmonary tuberculosis at a period in which percussion and auscultation will not permit a diagnosis of this disease. Since such cases are by no means of rare occurrence, and the sputum presents many other peculiarities which, as will subsequently be learned, direct the physician to a correct diagnosis, the semeiotic significance of the expectoration is not to be underestimated.

The above-mentioned examples suffice to indicate that the *macroscopic as well as the microscopic character of the sputum* should be considered during examination. The former reveals the gross composition of the sputum—the presence of mucus, pus, or blood; its amount and form and its odor and reaction; the other gives the essential elementary constituents and insignificant admixtures. Sometimes the macroscopic examination is of greater significance, sometimes the microscopic. Not infrequently the results of macroscopic examination render finer examination superfluous. Every careful examination of the sputum should, therefore, begin with thorough investigation of the *macroscopic characters.*

A reliable examination is possible only when the expectoration is received unmixed with other matter in a clean vessel. The ordinary glass sputum tumblers are preferable to the covered porcelain cups, because they permit rapid and ready observation of the quantity, color, and stratification of the sputum. In some instances it may be desirable to receive the sputum in a glass cylinder partly filled with water in order quickly to determine the form and gravity—*i.e.*, the air content—of the individual sputa. In general, it is best to secure the sputum alone—without the addition of water. The patients not confined to bed or house should be advised to carry with them the Dettweiler sputum glass.

After the expectoration has been inspected in the sputum glass, it is more thoroughly examined by spreading it upon a porcelain plate one-half of which is blackened with asphalt var-

nish. Small quantities only should be taken from the sputum glass, so that the secretion may be spread upon the plate in the thinnest possible layer. For examination the individual sputa should be drawn apart by two preparation needles (which, under certain circumstances, should not be made of metal) and the characteristic macroscopic features, subsequently to be described, carefully scrutinized. Each portion that has been examined is washed off and each new portion examined in the same manner.

Attention Should Generally be Directed to the Following Points:—

1. The **quantity** of sputum. This varies within wide limits —from occasional sputa to one or more liters in twenty-four hours. The largest amounts are observed in bronchorrhea, pulmonary abscess and gangrene, and in ruptured empyema. In the latter condition the amount expectorated may amount to four or five liters. In severe hemoptysis, also, the quantity is not infrequently quite large.

2. The **color**, which is dependent upon the admixture of mucus, pus, blood, and serum.

Accordingly there is obtained the important division of the expectoration into *mucoid, purulent, serous,* and *bloody* sputa, and—according to the nature and relative proportion of admixture—into *muco-purulent* or *purulent-mucoid, muco-hemorrhagic,* etc., sputa. The color of the sputum is lighter and more transparent the greater the content of mucus or water, and the more opaque the more richly cellular it is, whether the majority of cells present are red blood-corpuscles or pus-cells.

The most common forms of expectoration are the following:—

Simple mucoid sputum—**sputum crudum** of the ancients—is of glairy or grayish-white appearance and sometimes of liquid, sometimes of tenacious, consistence. According as it is raised easily or only after severe coughing its air content varies. It occurs *in every acute catarrh* of the upper air-passages and in bronchial asthma; also in chronic naso-pharyngeal catarrh, in which, however, it is of tougher consistency and sometimes mixed with dried scabs.

In the *muco-purulent expectoration*—the **sputum coctum** of the ancients—it must be determined whether it is of homo-

geneous character or whether its composition of mucus and pus can be recognized at a glance by the gross separation of these constituents. The first, *thoroughly mixed, muco-purulent,* yellowish-white sputum, in which the mucous content is in excess, is observed during the decline of every simple catarrh of the upper respiratory tract; the other is encountered in many cases of chronic bronchitis and especially in pulmonary phthisis. Here, however, the excess of pus is usually distinctly manifest; consequently the sputum is designated as *purulent mucoid.*

This occurs in two forms, the characteristic differences of which depend upon whether the pus coalesces or sinks to the bottom as separate, isolated, individual sputa. In the first case the *fresh expectoration* presents the gross composition of purulent "balled," yellow, or yellowish-green sputa and mucus; only after some time separation occurs; the pus sinks to the bottom and coalesces to a more or less homogeneous mass, while the mucous layer separates above into an almost colorless stratum, or else the thinner mucous layer is traversed by denser threads.

This type of sputum is most frequently observed in bronchiectasis and in bronchoblennorrhea, but also occurs in those forms of chronic pulmonary phthisis which are associated with severe general bronchitis, etc.

While this type of *purulent* mucoid sputum is not characteristic of any one disease process, the observation of the second is of more definite diagnostic significance. From ancient times "coin-shaped" (nummular) sputa have been looked upon as an important manifestation of phthisis. Such significance is also attached to it to-day, for this phenomenon is observed almost exclusively in this disease. The findings are most distinct when the contents of a cavity only is expectorated and the catarrhal phenomena are in abeyance. The nummular sputa are often of great volume, so that from nearly one-half to one tablespoonful is expelled by a single expectoration; their color is usually dirty yellow or yellowish green.

Pure purulent yellow sputum is most frequently expelled in pulmonary abscess and perforated empyema, but it also occurs in bronchoblennorrhea; the pus usually separates into two layers —an upper serous and a lower wholly purulent stratum.

Bloody expectoration occurs in bright-red and not rarely somewhat frothy form, in hemorrhages from pulmonary cavities or from aortic aneurisms which have ruptured into the trachea

or a bronchus, and mixed with mucus in the cases of foreign bodies in the air-passages. Bloody sputa grossly mixed with mucus or pus are regularly observed after subsidence of a severe phthisic hemorrhage. More uniformly blood-stained, purulent sputa occur in phthisic subjects with severe infiltration; on the other hand, occasional streaks of blood may be mixed with ordinary "naso-pharyngeal sputum" from the pharynx, especially when violent paroxysms of coughing are present. Dirty brownish-red sputum discolored by decomposition of the blood-coloring matter is observed in pulmonary gangrene. Of more frequent occurrence than the latter is the *thoroughly incorporated muco-hemorrhagic "rust-colored" rubiginous sputum observed in pneumonic patients,* which is of pathognomonic interest. When resolution of the inflammation is retarded, it sometimes assumes a deep-yellow or *grass-green* color, owing to the transformation of the blood-coloring matter, or it changes to a brownish to *prune-juice color* when the dreaded complication of (inflammatory) *edema* is added to croupous pneumonia. Pure bloody or tenacious mucoid sputum mixed with blood is observed in pulmonary infarction; it frequently resembles exactly the sputum of pneumonic patients.

Not infrequently the sputum presents a *raspberry-jelly-like* appearance in neoplasms of the bronchi or lung-tissue, and the same character of sputum is occasionally observed in hysteria (E. Wagner). [In March, 1903, I received a cylindric piece of tissue, 0.5×1.0 centimeter, expectorated by a male patient, aged 51 years, observed in the practice of Dr. F. F. C. Demarest, of Passaic, N. J. Microscopic examination showed the characteristic histologic structure of adenocarcinoma (bronchi), and a diagnosis was made accordingly. The sputum had a richly hemorrhagic character, and, although the physical signs suggested pulmonary tuberculosis, repeated microscopic examinations failed to reveal any tubercle bacilli. About two weeks after examination of the tissue cerebral metastasis occurred, followed by sudden death three months later.—Brooks.]

Purely *serous* sputum is a transparent, whitish fluid, and is characterized by its high albumin content. Owing to the difficulty with which it is expelled, it is often mixed with air in the form of large or fine bubbles. It is chiefly observed in ordinary

pulmonary edema, less frequently in the *"expectoration albuminеuse"* following paracentesis of the pleura, in valvular lesions, and in tumors of the chest-cavity. In *inflammatory pulmonary edema* it is more or less blood-stained, and then resembles "prune-juice" (see above).

3. The **tenacity** of the sputum is *chiefly due to admixture of mucus.* The sputum is usually extremely tenacious in pneumonia, asthma, and neoplasms. It is so coherent that it is often necessary to cut off the portions to be examined.

4. The **odor** is usually "stale"; in fetid bronchitis it is more or less offensive; in gangrene, putrid and fetid. According to Leyden, the pus discharged from a ruptured empyema often smells like *old cheese.*

5. The **reaction** is usually alkaline.

MACROSCOPIC EXAMINATION.

If the sputum be spread upon a plate in the manner above described, a number of peculiarities may be seen with the *naked eye.*

1. **"Rice Bodies" ("Linsen").**—These bodies—the famous *"corpuscula oryzoidea"* of the ancients—are found in the mucopurulent sputum of consumptives, and especially in and between the nummular masses at the bottom of the vessel. They are smooth, whitish-yellow, opaque, flattened or biconvex bodies varying from a pin-head to a lentil in size. They can readily be isolated by means of a needle or pincette and spread by slight pressure into a transparent layer between a cover-glass and slide. It is advisable to employ for examination only a small fragment no larger than the head of a pin. Small, mucus-covered crumbs of bread may be mistaken for these bodies.[1] Usually the difference can be noted when pressure is made upon the cover-glass. The genuine "rice bodies" can be crushed like cheese; the bread-crumb slides from under the cover-glass. *Dittrich's* plugs (see page 201) may also resemble "rice bodies." The microscope will decide the question. *The "rice bodies" are of great diagnostic value, because they contain elastic fibers and tubercle bacilli.*

[1] This error was made by Gruby, who described starch granules as "characteristic tubercle spheres." The mistake was pointed out by F. Simon (Virchow).

2. Fibrin Coagula.—These occur in almost every case of croupous pneumonia from the third to the seventh day of the disease: *i.e.,* during the stage of hepatization. They are slender, yellowish-white or yellowish-red threads, from two to three millimeters thick and one-half to several centimeters long. They are not infrequently decidedly *dendritic.* (The author found in a typic case of pneumonia a treelike branching coagulum twelve centimeters in length.) The shorter threads are seen on careful

Fig. 86.—Coagulum in Croupous Bronchitis. Natural Size. (Drawn After a Photograph.)

examination much more readily than the longer ones, because the latter are not rarely rolled upon themselves. By shaking with water in a test-tube the coagula can sometimes be found more easily. The number of coagula is very variable. It is not rare to find from twenty to thirty or more in twenty-four hours. Their fibrinous character is shown by their swelling and solution in acetic acid.

The coagula appear in a highly characteristic form, as **"bronchial trees"** (Fig. 86), in croupous or fibrinous bronchitis.

Under such circumstances they are usually few in number, but sometimes attain such a size that it may safely be concluded that a large portion of the bronchial tubular system is occluded. These treelike coagula are usually white, but are occasionally tinged whitish red. They most frequently appear in tubular form, less often as solid or flat, smooth formations. It is by no means rare for the trunk as well as the branches to show protrusions which are no doubt partly due to air. Occasionally the contents of the tubes themselves are bloody or mixed with blood; more frequently, however, they are filled with air. They are a constant accompaniment of the before-mentioned disease, which frequently occurs idiopathically or primarily, less often in diphtheria. They are easily overlooked by the inexperienced, *because* they *often* do not appear in the sputum as distinct coagula, *but rolled up in a more or less dense coil;* they are recognized by the experienced by their peculiar resemblance to meat-fragments. By shaking in water the coils can readily be unraveled and the branching, treelike coagulum brought into view. If on examination of the sputum neither these coils nor isolated threadlike forms are found, it is then advisable in every case of croupous bronchitis or pneumonia carefully to wash the sputum in a sputum glass.

3. Curschmann's Spirals.—In the glassy-slimy or tenacious-serous, muco-foamy expectoration of asthma patients, very rarely in other diseases of the bronchi, there are found, with more or less regularity, small, flocculent or fine, cylindric structures which are characterized by their grayish-white or whitish-yellow color and a spiral twisting or transverse marking which is frequently recognizable even with the naked eye. For a more detailed description see "Sputum in Bronchial Asthma."

4. Dittrich's Plugs.—In the yellow or greenish-purulent sediment of the sputum of fetid bronchitis and pulmonary gangrene (less often in chronic abscess of the lung and in phthisic sputum) there are usually found numerous whitish-yellow, smooth, pin-head to bean-sized granules, which can readily be "fished out" with a needle. They have an extremely offensive odor, are of cheesy consistency, and can readily be crushed. In addition to myriads of bacteria, common to the oral cavity, they contain numerous fat-crystals and occasionally monads.

5. Large Fragments of Tissue are found almost exclusively in *pulmonary gangrene.* They appear as grayish-yellow or discolored, occasionally distinctly black, shreds imbedded in mucoid pus. Their nature can be determined only by the microscope, since they usually consist only of a connective-tissue or, more rarely, of an elastic-tissue stroma.

6. Calcified Concretions, membranous remains of the echinococcus, etc., are rarely observed in the sputum. They will be considered in the appendix to this section (see page 216).

MICROSCOPIC EXAMINATION.

This leads to a satisfactory result only when a critical scrutiny of the sputum has previously been made, and it confirms many interpretations which a simple inspection of the sputum could render only probable.

In the microscopic picture there may be found:—

1. Red Blood-cells.—After a true hemorrhage these appear not only unaltered in form, but also in rouleaux. In the rubiginous expectoration they are seldom arranged in the form of rouleaux but more isolated side by side. In old sputum so-called red blood-cell "shadows" are also observed.

2. Colorless (White) Blood-cells (pus-corpuscles) constitute the majority of all of the cellular elements observed in the sputum. Their size varies and likewise their form. Almost all of them are multinucleated and the majority present *neutrophilic* granules; only in the sputum of asthmatics are *numerous eosinophile* and quite numerous *basophile* leucocytes regularly to be found (see page 162). W. Teichmüller ascribes to the increased number of eosinophile cells in the sputum of tuberculous subjects a favorable prognostic significance, a view which, however, has not remained uncontradicted.

The leucocytes possess the property of taking up in their cytoplasm various substances. Coal-pigment, altered blood-coloring matter, etc., are frequently observed within these cells. Furthermore, it is not improbable that the majority of the cells designated as "alveolar epithelia" are variously altered forms of leucocytes. The protoplasm very frequently shows fine or coarsely granular fatty metamorphosis, which is characterized

by the strongly refractive index. Other cells likewise present considerable coarse granulation; here, however, the spherules show a *decidedly dull* appearance, resembling that seen in crushed nerve-substance. For this reason they were designated by Virchow as *myelin droplets.* These large, dull spherules are also found outside of the cells. The shape of the cells containing them is sometimes round, sometimes ovoid, at other times somewhat polygonal. In addition to the droplets, one or several vesicular nuclei are visible (see Plate IX, Fig. 18).

Such cells can be seen in almost every sputum, even in sputum from the naso-pharynx of otherwise healthy individuals. Consequently the interpretation of these cells as alveolar epithelium has been and is now justly combated. Distinguished clinicians and pathologic anatomists (E. Wagner, Cohnheim) have strongly emphasized the unreliability of the data asserted in support of their epithelial character. Nevertheless, at the present time there appears to be a decided tendency to accept as correct the view that they are epithelial in nature. When discussing the so-called "heart-lesion cells," we will again refer to this mooted question.

3. Epithelia.—Corresponding to the various kinds of epithelium present in the different mucous membranes lining the respiratory passages, there is found in the sputum *squamous, cylindric,* and *ciliated* epithelium. The first is found in large numbers in naso-pharyngeal sputum (morning or choana sputum). Cylindric cells are often met with, especially in the first stages of *acute* catarrh of the upper air-passages and in severe paroxysms of coughing. Ciliated epithelium is seldom observed, but not so rarely as is generally stated. In the first days of acute catarrh (coryza and the like) and more so in a severe asthmatic attack, ciliated epithelium is frequently seen. The preparation must not be too hastily examined, because, as a rule, the ciliary movements can be observed only after the field has been watched for some time; furthermore, freshly expectorated sputa must be examined.

In the more chronic forms of bronchitis cylindric epithelium is rarely found, and ciliated epithelium almost never.

While the squamous epithelia almost always preserve their size and strongly refractive nucleus, the cylindric and cylindric ciliated present manifold morphologic changes. Sometimes they

are greatly swollen and glassy; sometimes they are distorted in shape and provided with more or less large, tail-like prolongations. In addition, their protoplasm is, as a rule, altered, more coarsely granular, fatty, etc.; the nucleus, however, is usually distinctly preserved.

The *"alveolar epithelia"* have been considered under "2." The author believes their *absolute* identification is extremely difficult. By these we usually understand the large, oval or round, also polygonal cells, three to six times as large as a white blood-corpuscle, which are found in almost every sputum. The usually large cell-body is coarsely granular and contains one or several "vesicle-like" nuclei. Very frequently the protoplasm shows the fine, highly refractive fat-droplets or dull translucent myelin spherules already described in connection with leucocytes. Not infrequently these are drawn out into peculiar shapes or have coalesced to form large droplets. The fat and myelin as well as the pigment granules taken up by the protoplasm are often so densely heaped together that the nuclei are obscured. We will refer to their origin and significance when discussing the so-called "heart-lesion cells" (see page 236).

4. Fatty Detritus, formed by the fatty degeneration of cells, occurs frequently in the form of very fine and somewhat coarser fat-droplets. They are found especially abundant when the sputum presents a purulent character. The *detritus* occurs, among other conditions, quite abundantly in pneumonic sputum, at the time of resolution of the exudate. It is of no especial diagnostic significance.

5. Elastic Fibers.—These are sometimes observed as isolated fibers, but are more frequently arranged in a delicate network (reticulum). They are distinguished from other similar elements, especially connective-tissue fibers, by their dark, sharp outline,—double contour,—their high refractive index, and their decided resistance to acids and alkalies. *The inexperienced are liable to mistake fat-crystal needles and foreign admixtures (wool and linen fibers) for them.* Fat-crystal needles liquefy and form fat-drops on heating, while the elastic fibers remain unchanged.

Under certain circumstances the elastic fibers may be derived from remnants of food remaining in the mouth; as a rule, how-

ever, these fibers are coarser and show neither the serpentine contour nor *alveolar* arrangement characteristic of those derived from the lungs (see Fig. 87).

The elastic fibers occur most abundantly in the above-described "corpora oryzoidea." In crush preparations made from portions of these bodies they are quite distinctly shown, usually without addition of acetic acid. If these bodies are absent, the different portions of the sputum must be examined and small particles, about the size of the head of a pin, removed from the dense, greenish-yellow masses and crushed between a cover-glass and a slide; or some 10-per-cent. potassium or sodium hydrate

Fig. 87.—Elastic Fibers from a Crushed Corpora Oryzoidea. × 350.

(caustic) solution may be added to the sputum. Should these methods not succeed after several preparations have been made, some of the sputum—a tablespoonful—must be mixed with an equal amount of 10-per-cent. caustic potash (or soda) and boiled until dissolved and then diluted with four times its volume of water and allowed to deposit a sediment in a conic glass. After twenty-four hours the supernatant fluid is poured off and some of the flocculent sediment taken for examination. The elastic fibers lose some of their sharpness of outline by this method.

Aside from *phthisis* and the rare ulcerative process of the upper air-passages as a result of syphilis, elastic fibers occur

principally in *pulmonary abscess,* and in *pulmonary gangrene.* In abscess they occur sometimes in small, white or grayish-yellow plugs or flakes of the tan-colored or purulent sputum, or—and this is, to a certain degree, characteristic—in long *tissue-shreds* which, in addition to many dense bundles, always show a delicate *alveolar* reticulum.

In **gangrene** the elastic fibers are occasionally absent, because they are dissolved (digested) by a trypsinoid ferment first described as present in this sputum by Filehne. Nevertheless, unquestionable exceptions have been observed by reliable authorities who, in addition to the *connective-tissue fibers* mentioned by Traube, detected dense tissue-shreds composed of *elastic* fibers in pulmonary gangrene. The author himself has repeatedly seen them in gangrene of the lung, and in a metapneumonic pulmonary gangrene he observed for about from eight to ten days the expulsion of grayish-black tissue-shreds from five to ten centimeters long, in which the *elastic fibers* were very well preserved. All that is necessary is to examine the sputum as fresh as possible. On the other hand, he has never seen elastic fibers in *bronchiectasis,* in the sputum of which condition they are said occasionally to occur, unless gangrene had supervened.

6. Fibrinous Coagula.—The coagula (Fig. 88) visible to the naked eye in croupous pneumonia and bronchitis (see page 219) show distinct *fibrin structure* on microscopic examination. They consist of delicate and coarse highly refractive fibrillæ, which are usually arranged parallel in dense bundles, not rarely intertwined to form a dense, feltlike structure, and surrounded by a greater or lesser collection of leucocytes. Red blood-cells are, likewise, frequently present in large numbers, and not rarely Charcot's crystals also.

The question raised by the fibrillary arrangement as to whether the appearance might not be due to closely approximated threads of ordinary mucus is decided by the fibrin reaction. If the threads dissolve by addition of acetic acid or are made more transparent, the diagnosis of fibrin is assured.

While pneumonia fibrin coagula can be quite readily teased out (broken up) and used for crush preparations, the firm, occasionally laminated coagulum of bronchial croup offers a greater resistance to these procedures. Usually it is possible only to break it up (tease) into

smaller and smaller clumps, which, however, are only in a few places generally sufficiently transparent to show that it is composed of a homogeneous, glistening, netlike stroma. By addition of acetic acid the clumps are made to swell (the minute, fibrillary structure, however, can be recognized only in sections made from the membrane which has been hardened in alcohol).

7. Curschmann's Spirals are shown in Figs. 94, 95, and 96. Their microscopic description will be given when discussing the sputum of asthma, in which they are almost *exclusively* found. Occasionally they are also found in the sputum of croupous pneumonia and bronchitis, as well as in pulmonary edema.

Fig. 88.—Delicate Fibrin Coagulum (from Croupous Pneumonia). × 350.

8. Crystals.—The crystals found in the sputum are: Charcot-Leyden crystals, fatty-acid needles and rosettes, cholesterin plates, and hematoïdin or bilirubin crystals. Much more rarely, tyrosin, leucin, and several others are found.

The **Charcot-Leyden** crystals (Fig. 96, *k*) are delicate, very sharply pointed octahedra, which occur in very variable size. They present a sometimes water-clear, transparent, sometimes a slightly yellowish-green, Rhine-wine-like color; they occur either isolated or in dense collections, which here and there are jumbled together, or in uniform rows, following the mucous shreds. They usually present well-marked pointed ends. In some crys-

tals a distinct transverse fissure is seen; others present at their margins or surface bulgings or a peculiarly undulated contour or the absence of a point. Others, again, show, instead of the smooth surface, finely granular inequalities, which indicate beginning disintegration. Some disintegration forms can be explained as derivatives of the crystals only by the grouping of dim droplets.

The crystals were first found in the sputum by Friedreich in croupous bronchitis. On the other hand, Leyden had drawn attention to their frequent occurrence in asthmatic expectoration. Since Charcot saw the same kind of crystals in the blood and spleen of cases of leukemia, the crystals have received the names of both investigators.

As has already been briefly stated, the crystals are of very frequent occurrence imbedded in the spirals in the sputum of bronchial asthma. They are by no means rarely observed in *fibrinous bronchitis.* The fact that the crystals occur specially in the old spiral formations renders it probable that they are in some way connected with "regressive metamorphosis of the round cells" (Curschmann). In the author's opinion, their development from *cylindric* (ciliated) *cells* is more probable. Salkowski's investigations are in harmony with this view. This authority, considering the optic (physical) and chemic characteristics of the crystals, concludes that they represent a *crystalline mucinoid substance.* The longer the asthmatic subject is free from paroxysms,—that is, the more time allowed for the formation of crystals,—the more densely the spirals are studded with these crystals. The fresh mucous coagula which have remained but a short time in the moist warmth of the bronchi show few or no crystals. That they, however, could have developed in them, also, is shown by the experiments of Unger, who, by allowing asthma sputum to remain in a moist chamber, was able to secure crystal-formation which was previously absent. Consequently the crystals which Curschmann justly designated as "accidental formations" otherwise resemble in every way the spiculate octahedra observed in the blood and spleen of leukemia as well as those in the stools. They are very unstable and difficult to preserve in preparations, but they preserve their characters for months in decomposing sputum. They readily dissolve in warm water, acids, and alkalies, but are *insoluble* in alcohol.

They may be *permanently preserved* by the following method: The thinly spread layer of coagulum containing the crystals is hardened in 5-per-cent. sublimate solution for about five minutes or for half an hour in absolute alcohol. Then stained in weak alcoholic solution of fuchsin (cleared in xylol) and imbedded in Canada balsam. Fixation of the air-dried preparation for one hour in absolute alcohol and subsequent staining with *Chenzinsky's* eosin-methylene blue solution (see page 152) also gives very good results.

Fatty-Acid Crystals (Fig. 89) occur chiefly in the form of *margarin needles*. These are delicate, transparent, usually very beautiful long, curved needles which are seldom isolated, and

Fig. 89.—Fat-crystal Needles *(a)* and Rosettes *(b)* and Masses of Cocci *(c)*. × 350.

usually appear arranged in dense broom or sheaflike bundles. Here and there they lie in groups and appear in reticular arrangement; so that they may give rise to confusion with elastic fibers, especially when their contour is very sharply defined and highly refractive. They are, however, *never dendritic* like the elastic fibers. If the object-glass be heated the needles rapidly dissolve. They then present *"distended"* places in their contour (where solution begins). By strong pressure upon the cover-glass such changes in form can also *be produced without previous heating*. Water and acids do not affect the needles; caustic alkalies dissolve them with difficulty. The needles are

completely dissolved by ether and warm alcohol. The crystals are constantly present in Dittrich's plugs (which see, page 201) in fetid bronchitis and pulmonary gangrene. They also occur in the small, yellowish fragments, which are expectorated by many perfectly healthy individuals by simply hawking, and have the odor and consistency of cheese. These formations are found in the stagnating secretion of the small mucous glands between the circumvallate papillæ and the epiglottis, as well as in the lacunæ of the tonsils.

Rosette fatty crystals (Fig. 90) are much more seldom found in the sputum. They appear as rosette-formed structures which may sometimes present great similarity to actinomycetes. They never form large agglomerations, however, occurring

Fig. 90.—Fat-crystal Rosette.

usually only as isolated small rosettes. They have a dull, yellowish color and are somewhat translucent. Heating of the preparation, ether, and alcohol rapidly dissolve them and at once identify them as fatty crystals.

Cholesterin (Fig. 91) occurs in the well-known, small and large rhombic plates, which are grouped together in large numbers superimposed and isolated, and not infrequently present notched and steplike margins. They are seldom observed in the sputum. They are most frequently observed in the pin-head- to lentil- sized, grayish-yellow masses seen in acute or chronic *pulmonary abscess.* The author twice observed these crystals in very stale-smelling, muco-purulent tuberculous sputum.

They are readily soluble in ether and hot alcohol; and *insoluble* in water, alkalies, and acids. On addition of sulphuric

acid solution begins at the margins and produces a reddish-brown glistening edge until the whole is transformed into a similarly stained drop. If some of Lugol's solution is first allowed to act upon them, the brownish crystals assume a bluish-red, green, and blue play of colors.

Hematoïdin Crystals (Fig. 92) occur in the form of brick-brown or ruby-red rhombic plates or columns and as delicate, bowed, similarly colored needles. The latter rarely are found isolated, but usually lie side by side or superimposed in groups. They frequently appear as though they were in immediate con-

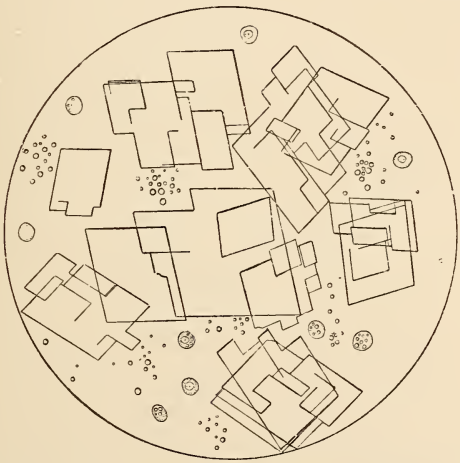


Fig. 91.—Cholesterin Plates. Crush Preparation. × 350.

tact with the platelets. They are seen radiating in the form of brushes or whisks from the four corners of the plates. As a rule, they are seldom observed. They are to be looked for in every case of *pulmonary abscess*. Here they are usually found in the gray or brownish-yellow granules, but also in the dense, yellow pus. The author found them once in a perforated empyema and once in a case of croupous pneumonia with retarded resolution. Here attention was at once directed to them by the peculiar *saffron-yellow* color of the purulent sputa. Ocher-yellow expectoration with numerous hematoïdin (bilirubin) crystals is found in perforation of hepatic echinococcus into the lung with

synchronous opening of the bile-ducts, less often in perforation of old pleuritic exudates. In such cases the sputum is characterized by a *gall-bitter* taste. The crystals are met with most abundantly in small, brownish masses found at the bottom of the sputum vessel.

The genesis of these crystals and their other features will be discussed when describing the characters of the sputum in cardiac valvular lesions.

Rare crystalline formations are the following:—

Tyrosin (Fig. 93) occurs in the form of delicate, glistening, colorless, conglomerate needles which usually form double whisks. They originate during proteid putrefaction induced by bacteria or ferments and are formed *only* in *old* pus-foci perforating into the lung (von Leyden-Kannenberg). That a certain period of time is necessary for their formation is shown by an observation made in von Leyden's clinic, in which tyrosin was absent in

Fig. 92.—Hematoïdin Crystals. Pulmonary Abscess. × 350.

rapid removal of the pus, while it was invariably present when the purulent matter had been retained (with exclusion of air) for some time.

Demonstration.—Some pus is allowed to dry upon a glass slide; the crystals which were previously in solution now develop in characteristic form, and can usually be very distinctly seen, particularly at the margins.

Tyrosin is readily soluble in hot water and ammonia and dilute hydrochloric and nitric acids, soluble with difficulty in acetic acid, and insoluble in alcohol and ether.

Leucin (Fig. 93) occurs almost constantly associated with tyrosin, but much less frequently than the latter. It likewise develops in connection with proteid decomposition by the action of unknown ferments in purulent sputum. It forms dull, glistening spherules which occasionally show a distinct radiate or con-

centric marking, and are readily soluble in hot water and dilute acids and alkalies, and insoluble in ether, and are thereby distinguished from large fat-drops.

Leucin can be demonstrated by allowing some of the pus for examination to dry upon an object-glass or by evaporating by gentle heat.

Crystals of *triple phosphate* (ammonio-magnesian phosphate) in the well-known coffin-lid form; *calcium oxalate* in the form of envelopes (found by Unger in asthma and by Fürbringer in diabetes); finally, *calcium carbonate* and *phosphate* may be mentioned as unusual constituents of the expectoration (see Section V).

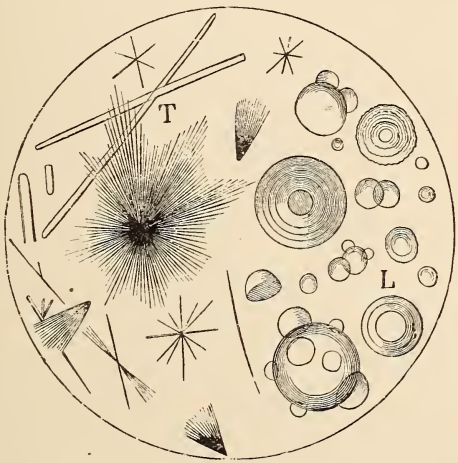


Fig. 93.—Tyrosin *(T)* and Leucin *(L)*. (After *Bizzozero*.)

VEGETABLE PARASITES IN THE SPUTUM.

Here the *leptothrix buccalis* and *thrush fungi (soor, aphthæ)* may be briefly mentioned, which may be present in the sputum as unimportant constituents. The morphologic features of these forms of fungi have already been described in Section I. If long, threadlike fungi are found in the sputum both of the forms just mentioned should first be thought of.

Very much less often there may be seen in the sputum the fungous elements belonging to the *aspergillus (fumigatus)* and *mucor (corymbifer)* which belong to the mold fungi already considered. These are

scarcely ever seen in the healthy air-passages, but many find location, although rarely, in diseased persons in whom decomposition processes in the lung-tissue have developed, as a result of tuberculous caseation, or in connection with pneumonia and hemorrhagic infarction.

In the sometimes more bloody-purulent, sometimes only muco-purulent expectoration, caseous fragments may attract attention. Microscopically there will be found in addition to elastic fibers the mycelia and fruit-hyphæ of molds.

Under certain circumstances attention should be directed to the elements of *actinomyces*. The simply muco-purulent, much less often bloody-mucoid, occasionally pure raspberry-jelly-like sputum shows here and there isolated *minute gritty, whitish, or greenish-yellow granules,* which on pressure under a cover-glass show—in addition to numerous dull or highly refractive coccoid bodies—wavy and in part branching and segmented threads with club-shaped ends. Typic actinomycetes (rosettes) with thick threads and clubs and elastic fibers occasionally also occur. Many fatty-degenerated leucocytes and fat-granule cells are always present. (For staining, etc., see page 84.)

In addition to these, a great variety of fission fungi whose admixture with the sputum must be recognized as wholly insignificant occur in the mouth. If it is desired to gain information in this direction, all that is necessary is to scrape the gums and back of the tongue with a spatula and examine microscopically the material thus obtained unstained. *Cocci, bacilli,* and *spirilla* will then be seen in active motion, which in the latter especially is effected by independent movements, in the others only by virtue of *Brownian* molecular movements.

In the section on bacteria it has briefly been mentioned that diplococci of the same nature as Fränkel's and Friedländer's cocci occasionally occur in the nasal and oral mucus of healthy individuals. This fact must be borne in mind when an examination of the sputum for *pathogenic bacteria* is undertaken.

The following pathogenic bacteria are of especial importance:—

1. R. Koch's *tubercle bacillus.*

It occurs "in pure culture" in the lentil-shaped necrotic plugs (masses), which show by their content of elastic fibers their origin from lung-tissue. Usually, however, they must be looked for in the yellowish or greenish-yellow pus. With very

few exceptions it can be found in every purulent or mucopurulent sputum from a tuberculous subject. More frequently bacillary examination of a chiefly mucoid sputum gives negative results. That bacilli occasionally occur in conjunction with [*micrococcus*] *tetragenus* has already been stated (page 54).

2. *Fränkel's pneumococcus* is of very frequent occurrence in the rusty sputum of pneumonia patients without offering any basis for differential diagnosis.

3. *Streptococci* and *staphylococci* are not uncommon. To the former, as is known, a certain etiologic rôle can be attributed in many forms of pneumonia (Weichselbaum); the others are occasionally observed in pus from abscess or perforated empyema.

4. The *glanders bacillus* should be looked for when peculiar affections exist in coachmen, hostlers, etc.

5. *Anthrax bacilli* are observed in woolsorters, ragpickers, etc., as concomitants of pulmonary mycosis.

6. *Diphtheria bacilli* are of importance only in so far as they occur in the expectorated membranes found in secondary croup.

7. *Influenza bacilli* are found quite constantly in the purulent sputum of la grippe patients.

The methods of demonstration and the peculiarities of the microbes are given in Section I.

Of the **animal parasites** there appear in the sputum echinococcic vesicles, distomum, and cercomonas.

The first are found as well-preserved vesicles or manifest their presence only by shreds of membrane or hooklets. They come either from the lungs, where they chiefly settle in the right lower lobe, or from a ruptured suppurated echinococcus, which, as a rule, is situated in the liver or more rarely in the pleura.

The sputum is always bloody in pulmonary echinococcus, or, when there is communication with the liver, bile-stained or ocher-yellow colored. The membrane-shreds are characterized by their uniformly white color and their tendency to curl (roll up) at the margins.

Microscopically there is seen in finely teased older lamellæ perfectly parallel striation, which is characteristic of *echinococcic membrane;* in the younger and more delicate formations this striation is usually indistinct. In such cases the diagnosis must rest upon detection of the *wreath of hooklets* and isolated *hooklets*, which are obviously positive evidence of the existence of the echinococcus.

The scolices are rarely found and only in an injured state; the hooklets, whose power of resistance is much greater, are oftener seen. For detailed description, etc., see page 120, Fig. 64. Not infrequently it is necessary to centrifuge the sputum.

The contents of the vesicles is a perfectly clear watery fluid which is free of albumin, but contains succinic acid and *sodium chlorid* (see "Aspirated Fluids," page 383).

Distomum Pulmonale (Bälz) usually manifests its presence in the air-passages by a slightly tenacious, mucoid, light or dark red expectoration in which the blood is present punctiform or in streaks or very decidedly in excess of the other constituents; severe hemoptysis is rare.

Microscopically there are observed in addition to white and red blood-cells and *numerous Charcot crystals*, unquestionable *parasite ova*, which can be recognized as brown points even with a hand lens. They are ovoid in shape and possess a thin, brown shell. For more detailed description see page 124, Fig. 67.

The occurrence of *cercomonas* in the expectoration derived from a freshly opened tonsillar abscess, as well as its frequent appearance in the sputum in pulmonary gangrene, has already been referred to (see page 106). Wagner occasionally saw similar objects in hysteric sputum (see page 242).

APPENDIX.

Pulmonary calculi (lung-stones) are very rarely observed in the sputum. They are expectorated in from lentil to bean size, and have a stony-hard consistence; they are sometimes smooth, at other times covered with small and large, blunt or spiculate processes, or are somewhat branched. They form in retained bronchial secretion, probably in small diverticula or depressions in the bronchial tube; rarer in the inspissated, stagnating contents of a cavity whose bronchus has become occluded. Calcification of the infiltrated tissues, which so frequently accompanies tuberculous cicatricial formation, may also lead to the formation of stony-hard, coarse concretions, which may gradually become loosened by disintegration of the surrounding tissues and be expelled by violent fits of coughing. Finally, exfoliation of *calcified bronchial glands* and their appearance in the sputum has been unquestionably observed.

If small fragments of such stones are scraped off and the reaction produced by addition of hydrochloric acid is watched under the microscope, there will invariably be observed distinct CO_2 formation—a proof that they are composed of calcium carbonate.

Many of the **foreign bodies** which enter the bronchi may be dislodged and expelled by fits of coughing. The sputum of such patients, provided the condition is acute, is usually bright, bloody, and foamy, but often assumes a distinctly fetid character. Aside

from a great variety of foreign bodies, fruit kernels and seeds, peas, rye-beards, blades of grass, etc., are especially worthy of mention. In a case observed by the author, a piece of calamus—which the patient had stuck in the cavity of an aching tooth to gain relief—entered the air-passage during the night and quickly excited violent symptoms of asphyxia, with profuse, bright bloody, muco-foamy expectoration. In another case a grain-beard, which had entered the bronchi, gave rise to intermittent fetid bronchitis for a period of ten years. Rye-beards frequently excite pulmonary abscess. Israel saw pulmonary actinomycosis develop from aspiration of a fragment of a tooth (see page 83). Out of a gangrenous pulmonary cavity operated upon by the author several foul-smelling *tooth-fragments* were expelled through the fistulous tract.

CHARACTER OF THE SPUTUM IN CERTAIN DISEASES.

In **acute catarrh of the air-passages** the amount and character of the sputum depend upon the severity and extent of the disease. In the first few days the secretion is usually transparent and watery, as in an ordinary cold, and becomes thicker and more tenacious and slimy in the second, or "resolution," stage *(sputum crudum et coctum)*. The viscidity varies with the amount of mucus, which is positively demonstrated by coagulation on addition of acetic acid. *Microscopically* there will be found, in addition to transparent mucous shreds, variously shaped epithelial cells, especially numerous cylindric and goblet-cells, in a more or less advanced stage of mucoid change, as well as large numbers of round cells which present features already described. The thicker and more opaque the secretion, the greater the number of pus-cells.

In **chronic catarrh** the expectoration is almost constantly muco-purulent in character. The amount is sometimes slight,—*e.g.,* in spite of severe, aggravating cough in *"catarrhe sec"* (Laennec),—sometimes remarkably profuse. The latter is the case in *bronchoblennorrhea,* which depends upon acute exacerbation of a chronic catarrh and is excited through "colds," inhalation of mechanically or chemically irritating substances, or—what is most probable—by infectious or toxic influences aris-

ing from decomposition of the secretion. The sputum is then expelled in large amounts—one-half to one liter and more. The individual muco-purulent sputa are voluminous and are usually easily expectorated. As a rule, distinct *stratification* occurs, the pus settling to the bottom and the mucus collecting above in a cloudy, sero-mucoid layer. In many cases the latter further separates into two layers—above the pus stratum there is a serous layer and above that a muco-foamy stratum.

While here large amounts of sputum can be raised without much difficulty, expectoration in *"pituitous" catarrh*, also called *blennorrhea serosa*, is accompanied by severe asthmatic disturbance. The sputum is also very profuse,—one to one and one-half liters in twenty-four hours,—*but far less richly cellular*, more sero-mucoid *(asthma humidum)*. Sudden exacerbations of chronic bronchitis (following severe colds) give rise to the development of this condition; also the ordinary contracted kidney is said to favor its occurrence (Strümpell).

The sputum of **bronchiectases** occupies a special position.

As is known, these develop in cylindric and sacculated form as a result of frequent acute exacerbations of chronic bronchitis, or as the result of contractile processes occurring in the neighborhood of the bronchi, whether these are due to cicatricial adhesions of the pleura or to inflammatory thickening of the interlobular connective tissue exerting pressure and traction upon the lumina of the bronchi and preventing uniform distribution of the respired air. Stagnation of the usually large amount of secretion elaborated by the inflamed mucous membrane produces further aggravation. The mucous membrane is partly atrophic, often greatly thickened; the epithelium is usually altered; in addition to well-preserved cylindric cells, there are chiefly found epithelia in an advanced stage of mucoid degeneration and deprived of their cilia. Putrid decomposition of the bronchiectatic contents may lead to ulcerative processes resulting in the development of an ulcerating cavity.

The expectoration is characterized by the fact that the individual sputa are very voluminous and are expelled in large amounts and in quick succession by slight efforts of coughing or pressure movements (consequently *"mouthful expectoration"* is spoken of); it is thin, muco-purulent, usually "insipid" in odor and only occasionally offensive, namely: when undergoing decomposition. It usually separates into a purulent and muco-serous layer; less often into three layers.

In addition to pus-corpuscles (which are usually decidedly fatty), mucoid degenerated epithelia, and countless putrefactive bacteria, *margarin crystals* are of not infrequent occurrence.

Much more rarely *leucin* and *tyrosin* are found in evaporated or dried preparations. The author has never seen *elastic fibers* in this sputum; admixture of blood is not infrequently met with.

Decomposition of the bronchiectatic secretion gives rise to **fetid, or putrid, bronchitis.** The sputum produced in this affection is usually very abundant, dirty greenish or more grayish green in color, usually liquid, and extremely offensive in odor. On long standing it always separates into *three layers*, the uppermost of which is dirty muco-foamy, with many villouslike prolongations reaching into the middle, discolored, greenish-yellow, fluid layer, while the lower layer is densely purulent. In the latter are suspended the usually foul-smelling **Dittrich plugs,** the form and composition (fat-needles, bacteria, etc.) of which have already been described (page 201).

The sputum of **fibrinous bronchitis** presents a decidedly characteristic appearance. This disease in its primary form is chiefly observed in young or middle-aged subjects, and about twice as often in males as in females. It is seldom observed and then usually as a chronic affection which is subject to exacerbations at very variable intervals. There is not much doubt that many, if not all, cases of *acute* fibrinous bronchitis are caused by *diphtheria*. The author saw five such cases in which bacteriologic examination confirmed the diagnosis. The sputum is usually profuse, mucoid-foamy, and contains as essential constituents the croupous branching coagula, already described (pages 200, 206). These are sometimes recognized at once, but more frequently they are concealed in the sputum. As their expectoration often requires great effort, the sputa are usually tinged with blood. In addition to the coagula, *Charcot-Leyden* crystals are not infrequently observed; less often *Curschmann's spirals* and granular blood-coloring matter and still more rarely ciliated epithelia are seen. As croupous bronchitis is occasionally observed in tuberculous subjects, it is advisable also to examine for tubercle bacilli.

The **sputum of acute croupous pneumonia** varies in character according to the different stages of the affection.

We distinguish in lobar pneumonia three stages according as the affected pulmonary area shows more or less intense hyperemia (congestion), or the formation of exudation in the alveoli and bronchioles, or,

finally, resolution of the exudate. In the beginning the capillaries are greatly distended with blood; the exudate is at first more serous, subsequently mingled with great numbers of leucocytes, red blood-corpuscles, and numerous fine and coarse, usually sharply defined, fibrin threads. In the stage of resolution, the red blood-corpuscles appear decolored, the leucocytes in great part fatty degenerated, and the fibrin in an advanced state of disintegration.

At the outset of the disease the sputum is scanty, very tough, and sticky, and yellowish-red in color. In a moderately thin layer it is transparent. Owing to the viscid character of the sputum, it can be expectorated only with difficulty. **Microscopically** it consists of mucus precipitable by acetic acid, red blood-corpuscles usually arranged side by side, and fresh and old (somewhat granular) round cells.

In the *second* stage the *rust-colored (rubiginosa)* or *saffron-colored (crocea)* or more bloody-mucoid *(sanguinolenta)* sputa coalesce to form a thoroughly incorporated tenacious mass, which adheres to the sputum vessel and can be separated only with a needle or scissors. If the vessel is tilted, the sputum often remains sticking to the sides, or the whole mass glides out very slowly. Owing to the difficulty with which the sputa are expectorated, they contain numerous large air-bubbles. Corresponding to the fibrinous exudate now poured into the alveoli, they present small, light-yellow, fibrinous clumps or the above-described *fibrin threads,* which are not rarely single or branching. These fibrin threads are easily differentiated from *Curschmann's spirals,* which are also occasionally observed here.

In addition to the characteristic rust-colored sputum muco-purulent streaks and flocculi are also observed as a result of the accompanying bronchial catarrh.

With resolution of the exudate (third stage) progressive decoloration of the sputum occurs. From day to day it grows less yellow in color, becomes muco-purulent, adheres slightly or not at all to the wall of the sputum vessel, and may be more readily poured out in separate parts. The total mass increases and the coagula disappear. *Microscopically* there are found chiefly round cells, far advanced in fatty degeneration; also granular cells and smaller and larger drops of free fat, isolated or collected in small clumps.

Many *deviations* from the appearances of the sputum here described are not infrequently observed without any apparent explanation.

The rust-colored sputum may be replaced by that of a *decidedly bloody* character. This occurs in the so-called traumatic (contusion) pneumonia and in drunkards. In the pneumonia accompanying congestion of the lesser circulation the amount of blood in the sputum is also pronounced, but the quantity of mucus and consequently the tenacity is less marked. The color (at first brick-red) and character often subsequently approach that seen in inflammatory edema; or only occasional sputa are expectorated without the prognosis being altered in any way thereby, unless the deficiency of expectoration is due to great general weakness of the patient. Very cloudy, opaque sputum is present from the very first in pneumonia occurring in people suffering from pre-existing bronchial catarrh. In such cases rust-colored admixtures can be observed only on careful examination.

The croupous *pneumonia in la grippe* also presents a similar appearance. From the first the expectoration here consists of muco-purulent or even purulent-mucoid, often lumpy sputa, which but rarely possess a pale-rose or rusty shade of color.

If with the pneumonia there is co-existent icterus catarrhalis the sputum often shows a distinct *greenish* shade, owing to the admixture of bile-coloring matter, which has passed from the tissues into the sputum and which can readily be detected by the well-known reactions.

Pronounced *grass-green staining* is also observed in *delayed* resolution of the pneumonic exudate. As a rule, this discoloration is of no further prognostic significance. In a number of irregularly progressing cases, however, this discoloration precedes *abscess-formation*, to which Traube has drawn attention (and according to the author's experience, justly so). In both instances the *green staining* is "referable to transformation of the blood-coloring matter dissolved in the sputum under the influence of oxygen," as is also to be seen in the rusty, yellow, and green discoloration occurring in the blood-spots in bruising of the skin.

In delayed resolution unusually intense *saffron-yellow* expectoration is occasionally observed. The author has already stated that he has seen in one case of this character beautifully

formed *hematoïdin crystal plates and needles.* There was no reason to suspect the presence of an abscess.

In *pneumonia of children* there is usually no sputum to be observed, because it is generally swallowed. That which is expectorated, however, often shows, even in genuine lobar pneumonia, a simple catarrhal character, and is not rust colored. A similar appearance of the sputum is not rarely observed in *pneumonia of drunkards* (topers), of the *aged,* and of the *insane.*

The unfortunately not rarely fatal termination of croupous pneumonia often occurs as the result of **inflammatory [pulmonary] edema,** which is accompanied by characteristic expectoration.

The sputum is decidedly liquid, dark brown, sero-mucoid, foamy, *prune-juice-like (jus de pruneaux,* Andral), and profuse. Between the sero-foamy portions there may often be seen more tenacious sputa derived from the genuine croupous inflammation, which stick together in a firm, coherent mass and float in the brownish-red fluid. *Microscopically* there are found in the latter, in addition to red blood-corpuscles, only a few cells, while in the former, after "teasing," there are seen fibrin coagula and numerous colorless blood-corpuscles (leucocytes). Addition of acetic acid produces only a slight precipitate in the preparation taken from the fluid portion, while, on boiling, a more or less thick coagulation is obtained, indicating the presence of a large amount of albumin.

The sputum just described always suggests a serious, if not a fatal, prognosis. Termination in pulmonary gangrene or abscess is very rare.

In **gangrene** the sputum presents a certain similarity to that just described. It is by no means rare to observe, on the advent of pulmonary gangrene, that the previously tenacious rubiginous sputum becomes more fluid and brownish in color, without at first presenting the putrid, stinking, pathognomonic character. *Microscopically,* in contrast to the preceding form of expectoration the red blood-corpuscles are almost wholly destroyed; so that the majority of them are in the form of "shadows." In addition there may be seen small and large *tissue-shreds,* which are usually found in the blackish fragments present in the sputum.

As already mentioned, the sputum very soon becomes extremely offensive in odor. The brownish-red color gives place to a more dirty-greenish or green shade; the quantity is considerably increased (one-fourth to one-half liter in twenty-four hours); the consistency is diminished, usually fluid. As a rule, separation into *three layers* rapidly takes place. The uppermost, dirty-gray layer contains tenacious mucous masses, mixed with air, which partly project in villous form into the broad, dirty-greenish liquid layer. The lower layer, which is of variable depth, is composed of thick, coherent pus in which, *in addition* to the already described Dittrich plugs, dirty grayish-yellow, irregularly fissured fragments are imbedded. *Microscopically* these are usually found to be delicate connective-tissue shreds, which swell up in acetic acid; they are surrounded by myriads of bacteria and fatty *detritus,* fat-drops, fat-needles, and dark-blackish pigment and hemosiderin. Hematoïdin crystals are very seldom found, however. *Elastic fibers,* which are often absent, undoubtedly occur in pulmonary gangrene, as has already been mentioned.

The bacteria occurring in the sputum at once remind one of the appearance which can always be observed in the secretions of unclean mouths. In addition to large numbers of cocci there are seen bacilli, spirilla, and the leptothricial threads belonging to the algæ; more rarely, also, cercomonas forms. The same flora are observed in the fragments taken fresh from gangrenous cavities opened for therapeutic purposes.

Of diagnostic importance is the occurrence of *pseudo-tubercle bacilli,* which can be distinguished from Koch's tubercle bacilli only by careful staining (see page 53 *et seq.*).

From this description it is seen that the sputum of gangrene resembles that observed in fetid bronchitic expectoration. Indeed, the resemblance may be such as to render the diagnosis doubtful. In such instances the detection of *tissue-fragments,* which may or may not contain elastic fibers, will decide the question. Expulsion of tissue *never* occurs in fetid bronchitis, but is the rule in gangrene. Not infrequently fetid bronchitis of other portions of the lung is associated with gangrene.

Pulmonary gangrene occasionally follows croupous pneumonia; whether it is usually the genuine croupous form or not

appears to the author to be very doubtful; more probably it is preceded or accompanied by the aspiration of foreign substances. Large foreign bodies (pieces of bone, fish-bones, fruit-stones, buttons, etc.) may give rise to pneumonia and gangrene even after long lodgment. Furthermore, pulmonary gangrene may develop in fetid bronchitis and in suppurative and putrid processes occurring in the neighborhood of the bronchi. Finally, embolic processes in septic conditions and injuries of the thorax may result in gangrene.

The principal characters of the sputum are the same in all cases, namely: offensive odor, fluidity, pus mingled with tissue-shreds and fragments, with a constant tendency to separate into *three* layers.

The appearance of the sputum is different in **pulmonary abscess.** Here the expectoration presents a purely purulent character and a slightly stale odor. It is profuse, especially at the outset, occasionally being discharged to the amount of from one-half to one liter in twenty-four hours. On standing, separation into *two distinct layers* occurs: below the slightly greenish-colored, liquid upper layer, there is deposited a uniform coherent mass of pus in which, when spread out, large grayish-yellow, irregularly serrated or somewhat smooth shreds or small, yellowish-white or blackish granules and yellowish-brown flocculi can sometimes be found.

Microscopically the shreds show elastic fibers arranged in alveolar form and dense and but slightly tortuous threads; there are also found a great number of micrococci, especially the *staphylococcus pyogenes aureus,* fat-crystals in the form of needles and rosettes (see page 209), and decidedly fatty pus-cells. Finally beautiful hematoïdin crystals in needles and plates are not rarely found in the brown flocculi.

If the abscess follows croupous pneumonia, the expectoration is often mixed with blood, and then has a *chocolate-brown* color.

The sputum occurring in *chronic pulmonary abscess* presents a similar character. The pus is profuse, but usually contains no tissue-shreds or only microscopic evidence of disintegration (elastic fibers). Hematoïdin crystals do *not* occur, but von Leyden has occasionally found *cholesterin.*

It is plain that **the sputum in perforation of empyema** must possess great similarity to that described above. The sputum is likewise purulent and intermittently discharged, but always much more profuse (as high as from four to five liters in twenty-four hours), and presents the same stratification and almost the same microscopic characters. As a rule, however, the tissue-shreds are absent and elastic fibers are only occasionally observed. Fat-crystals, etc., are present. That *tyrosin* is observed in old suppurations in the pleural cavity which occasionally freely communicate with the bronchi has already been mentioned.

In view of the fact that *actinomyces* may give rise to multiple abscesses in the lung-tissue, a search for the *ray-fungus granules* should be made in every case of pulmonary abscess, the origin of which cannot otherwise be explained. They appear as yellowish-white grains, about the size of a milletseed, the microscopic appearance of which is figured on page 83.

The glanders bacillus should also be sought for. That leptothrix and cercomonas may occur in the pus of an abscess-cavity in the absence of any proof of their pathogenic connection with the process has been referred to on pages 94, 106, and 216.

Finally, it must be remembered that purulent sputum may be indicative of rupture into the lungs of an *echinococcic cyst* of neighboring parts. When a communication with the bile-channels exists, the sputum presents a distinctly *ocher-yellow* color, has a gall-bitter taste, and gives a distinct reaction for bile-coloring matter. *Microscopically* there are found, in addition to the shreds and hooklets derived from the parasite, beautiful hematoïdin or bilirubin crystals. In a case observed by the author there were also present numerous *cholesterin crystals* in addition to shreds of membrane.

The author would especially emphasize that he also once observed an ocher-yellow sputum in a chronic ocher-colored pleural exudate, which occasionally communicated with the bronchi and permitted the escape of a peculiar, glistening fluid, rich in cholesterin (see page 210). Echinococcus was certainly *not* present.

The Expectoration in Pulmonary Tuberculosis.—Although every effort has from the earliest times been made to determine

certain features as characteristic of phthisic sputum, observers have become more and more convinced of the unreliability of such evidence in exact diagnosis. **The only positive proof of the existence of tuberculosis is obtained by staining the tubercle bacilli contained in the sputum.** All other features of the sputum have but a relative value, since nontuberculous subjects may expectorate an exactly similar sputum. Nevertheless, the expectoration of tuberculous individuals or those suspected to be tuberculous demands careful consideration for many reasons.

The sputum is muco-purulent or more purulent-mucoid, and quite thoroughly incorporated. The former occurs when no decided destruction of tissue has as yet occurred and is wholly uncharacteristic; on the other hand, the second variety, which occurs with the presence of *cavities,* presents typic features which are worthy of more detailed description. This sputum is characterized by more or less numerous "balled," large globular, distinctly purulent masses which present a roughened and ragged surface. When deposited upon a smooth surface they spread out into an almost round form and are therefore designated as *nummular sputa.* If they are expectorated in a vessel containing water, they often sink quickly to the bottom *(fundum petens);* others, prevented from sinking by the presence of mucous shreds, remain upon the surface and float there as ovoid or spheric masses *(globosum).* In the latter masses, particularly, the fissured character of cavernous sputum can plainly be seen. The densely globular, almost air-free character of such sputum permits the conclusion that it originated in a cavity, since otherwise a large amount of air would certainly be included by gradual formation of such globules in the bronchi. In addition to this globular sputum, however, large amounts of muco-purulent masses often occur, which obscure the characteristic features. Furthermore, similar globular sputa are formed in other and nontuberculous cavities, especially in sacculated bronchiectasis.

It is by no means rare for these otherwise isolated nummular sputa to coalesce. Under such circumstances it is quite difficult to make a diagnosis from the macroscopic appearance of the expectoration, for there is then scarcely any difference between the tuberculous expectoration and that present in severe diffuse bronchitis or bronchiectasis.

Of great value in such cases is the **frequent admixture of blood.** As has already been mentioned, this blood not infrequently occurs free. It sometimes occurs only in the form of single or several pure bloody sputa (hemoptysis), sometimes in large amounts, seldom exceeding one-half liter (hemoptysis). More frequently it is mixed in clumps or streaks with the mucopus or is more thoroughly incorporated with it, so that the sputum has a chocolatelike appearance. All of these varieties of small and large hemorrhages are certainly deserving of careful consideration, since experience has shown that tuberculosis especially favors the frequent expulsion of blood, whether the blood is derived from large vessels traversing the cavity or from such located in the walls which are eroded by the advance of disintegration or escapes as the result of gradual *"diapedesis."* Not rarely, however, the frequent admixture of blood has *led to error.* Consequently, in obscure cases it should be remembered that exactly similar sputa may occur in other affections. *Neoplasms,* which will later be considered; *echinococcus* of the lung, which *always* gives rise to bloody expectoration; *actinomycosis, hysteria,* etc., favor the occurrence of bloody sputa in many forms. It should also be borne in mind that *ulcers* of the larynx and bronchi (syphilis) and many forms of hemorrhagic diathesis, etc., may lead to pulmonary hemorrhage.

The detection of **corpora oryzoidea** *("Linsen")* is of great importance. It has already been stated that they are characterized by the large amount of elastic fibers, arranged in alveolar form, and of bacilli in "pure culture" which they contain. *Their demonstration permits the positive assumption of a destructive (caseating) process in the tissue of the lung.*

Information as to the origin and significance of these smooth, yellowish-white, opaque plugs can best be obtained at the necropsy of a tuberculous subject whose lungs are filled with cavities, by carefully examining the contents and walls of the latter (excavations). Scarcely such a case would be found in which these bodies are absent. Often six to ten and more are found in a single cavity. They usually lie perfectly free and movable upon the wall; sometimes they are still partly adherent to the same. They are especially apt to be located in the small depressions which are almost always present in every cavity-wall. Even with the naked eye the absolute identity of these bodies with the "Linsen" present in the sputum is unmistakable. Their identity is fully established by microscopic examination.

This condition reveals the great value of their detection, as Virchow showed long ago (in January, 1851). They are not often met with. Although such bodies *can* be found in the majority of patients in whom pulmonary cavities exist, the search of the sputa often requires much time. As a rule, the destruction of tissue can be determined by the pulmonary physical signs and the tuberculous nature more quickly established by the demonstration of tubercle bacilli.

Isolated elastic fibers are not infrequently met with, if any of the greenish portions of the pus are examined under the microscope. Their detection is facilitated by the addition of 3-per-cent. caustic soda to the preparation. If this does not succeed, boiling with caustic soda is necessary (see page 51). Confusion with fat-crystals can certainly be avoided (see pages 109 and 110).

The tuberculous character of the sputum is assured only by the **demonstration of tubercle bacilli.** Tuberculous sputum is distinguished from all other kinds of expectoration by the presence in it of specific bacilli whose rôle as exciters of tuberculosis has been unquestionably proved. *For this reason examination for bacilli is of the utmost importance.* In it we have a means of determination which far excels all other formerly known methods. *"The tubercle bacilli are not only a cause of tuberculosis, but the only cause of it; without tubercle bacilli there is no tuberculosis."* These words of Koch hold good to-day, and the many years of investigation which have passed since the appearance of his communication (1882) have repeatedly confirmed the correctness of his statements. The rare instances in which the most careful examination fails to show the bacilli in the sputum, in spite of the existence of tuberculosis, cannot invalidate them. Compared with the enormous majority of positive findings secured, these cases simply teach that negative results of one or several examinations of the sputum do not justify one in positively excluding the presence of tuberculosis.

The greatest practical value of the examination for bacilli is in cases in which a suspicion of tuberculosis exists, but cannot be confirmed by physical examination. In such instances the demonstration of the bacilli in the often scanty sputum settles the diagnosis at once; and in not a few cases of another kind—*e.g.*,

in those forms appearing under the guise of an acute croupous pneumonia—examination for the bacilli is of decisive importance in diagnosis and prognosis.

The *form* and *number of bacilli* occurring in the sputum may be briefly outlined. In Section I it has been stated that the bacilli found in the sputum very frequently present within their bodies clear spaces, which were formerly interpreted as spores. Whether or not this view is correct and applicable in all instances is very questionable. The number of bacilli often depends upon the extent and severity of the pathologic process; hectic-fever patients usually expectorate numerous bacilli. Exceptions unquestionably occur, however. There are seriously affected subjects who present a sputum containing but occasional bacilli, and nonfebrile individuals in good physical condition who expectorate considerable numbers of them. Such exceptions should be borne in mind before a prognosis is expressed; moreover, the other clinical features must also be considered.

The occurrence of **pseudotubercle bacilli** [smegma bacilli] in the sputum is worthy of attention. In doubtful cases the greatest care should be taken in staining in order to exclude this source of error (pages 53 and 54). Furthermore, the author would here emphasize the necessity of employing *new* cover-glasses [or slides] and scrupulously cleansed examination dishes and sterilized needles [by heating to redness in flame].

Finally, the changes which the sputum undergoes during the *employment of tuberculin* may be briefly considered. According to numerous concurring reports, the purulent-mucoid sputum of tuberculous subjects assumes a more mucoid character after frequent subcutaneous injections of tuberculin, and the number of bacilli is decidedly diminished. The latter not infrequently present evidence of degeneration and occur in large groups more often than is otherwise the case in the sputum. It is not rare for the sputum to be tinged with blood.

The **sputum in bronchial asthma** is shown in Figs. 94, 95, and 96. The amount of sputum expectorated during the paroxysms varies within wide limits; sometimes only one to two tablespoonfuls, sometimes as much as one-half to three-fourths liter is expelled. The grayish-white, extremely tenacious, mucoid sputa coalesce to form a homogeneous mass which is covered by a layer of foam resembling the beaten white of egg. If an attempt is made to pour out a portion of the sputum, the whole mass usually comes away. The portion to be examined must, therefore, be separated with a needle or other apparatus. The character of the sputum varies according as the paroxysms are

violent and of only one to two days' duration, or the dyspnea continues for months with either frequent or occasional exacerbations. In the first instance the expectoration, which may sometimes rapidly increase to one-half liter or more, usually ceases after several days, while in other cases the amount varies between fifty and one hundred cubic centimeters, and is rapidly augmented only during the paroxysm. Here, also, the grayish-white color and viscid character of the sputum is preserved. On long standing the asthmatic expectoration becomes more fluid and not infrequently assumes a grass-green tinge.

Fig. 94.—Curschmann's Spiral. × 110.

If small divided portions of the sputum are spread upon a black plate, there will be found, in a majority of cases, lying beside and between the grayish-white—seldom pus-streaked—balled sputum masses and mucous shreds peculiar sagolike, transparent formations *(Curschmann's spirals)*. These are partly gray clumps, here and there flecked with yellow; partly grayish white, transversely striated, or spirally twisted shreds, one-half to one and one-half millimeters thick and from one-half to eight centimeters long.

Microscopically, these bodies, which are often fixed with difficulty under the cover-glass, appear as delicately twisted spirals of glassy transparency. At their extremities there can frequently be observed the manner in which the numerous twisted fibrillæ which compose the spiral separate and unite. The thread, composed of numerous different fibrillæ, is surrounded by a transparent mucous sheath which is often studded with numerous round, caudate, and delicate, spindle-shaped

cells. In addition to the spiral arrangement of the individual fibrillæ, many coarser twists as well as knotted and loop formations are noticeable in the course of the thread (Figs. 94, 95, and 96).

In many spirals there is at once noticed a uniform delicate, glistening, white thread, which occupies exactly the axis of the structure, and is only here and there interrupted in its course by a sharp twist (knotting) in the spiral. In Fig. 95 (drawn from a photograph) it will be seen that a peculiar spiral winds around the clear axis interrupted by a number of nodules, and that this central portion is inclosed in a larger spiral (mantle spiral).

This portion of the spiral, which Curschmann designated as the *central fiber*, is apparently in a majority of cases to be considered as an

Fig. 95.—Curschmann's Spiral with Central Thread.
× 110.

optic expression of the mucous spiral, much less frequently as a separate body composed of a homogeneous substance, or of delicate, twisted fibrillæ. Such a central fiber can be artificially produced by fixing one end of a mucous thread upon a glass slide and twisting the other end by means of a pair of forceps (Sänger).

It is by no means rare, especially in the first asthmatic paroxysm recurring after a long pause, to find *yellow-flecked or more uniformly yellowish, somewhat granular, dense threads* in the sputum which microscopically show, in addition to often indistinct spiral twisting, dense clumps and streaks of delicate Charcot crystals beneath the masses of round cells composing the mantle of the spiral. Aside from their bright color, *these crystal-*

laden spirals are often recognized by a distinct grating when crushed between the cover-glass.

Some of these yellow, barley-grain-sized bodies are so densely filled with crystals that the delicate spiral markings can no longer be observed. Nevertheless, from the whole macroscopic impression, it may with a certain degree of probability be concluded that they are altered Curschmann spirals. Curschmann long ago expressed this view and designated the formation of crystals as "age phenomena," especially as the appearance of the yellow-crystal-laden forms is observed particularly in the first paroxysms occurring after long intervals. In support of this view the author can present the following case:—

A man, 27 years old, comes to my Leipzig clinic for asthmatic trouble which had existed for two days. The distinct expiratory

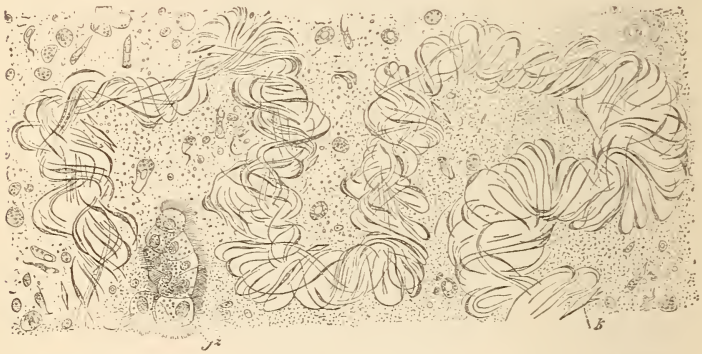


Fig. 96.—Curschmann's Spiral, Composed of Delicately Twisted Mucous Threads and Spindle-shaped Cells. × 350.

In the periphery normal and swollen cylindric and ciliated cells (*fz*); also eosino- (or baso-) phile cells and Charcot-Leyden crystals (*k*). The latter, however, are from other parts of the expectoration: *i.e.*, from another field of the microscope.

dyspnea and the habitus of the thorax indicate asthma. In a flake of sputum which the patient coughs up are imbedded several yellow plugs which grate on crushing under a cover-glass. Administration of potassium iodide is followed in the next few days by profuse expectoration of an extremely viscid, confluent, *saffron-yellow* sputum, in which even to the naked eye the yellow color permits the assumption of the presence of numerous, almost sulphur-yellow granules, and *wheat-grain-sized* masses, imbedded in the extremely tenacious mucoid matrix. Distinct pus-markings are absent. All the flocculi show on *microscopic* examination great numbers of minute and strikingly large octahedra in addition to extremely numerous eosinophiles and relatively numerous mast-cells. *The patient formerly had frequent attacks, but for a year and a half has*

not had a single paroxysm. Rapid improvement followed, with complete disappearance of the yellow masses; distinct spirals were observed one year later, when the patient had several mild attacks and again visited the clinic.

The manner in which the spiral twisting of the mucous coagula occurs can only be conjectured. Curschmann attributes the twisted form to the spiral mode of anastomosis of the finer with the coarser bronchial branches, as demonstrated by F. E. Schultze. A. Schmidt explains their occurrence through the agency of the spiral movements of the expired air. There can be no doubt that the formations as we see them in the sputum are produced in the bronchioles, for A. Schmidt found them in these as well as in the smaller bronchi of a patient who died during an *asthmatic attack.* No spiral formations were found in the mucus present in the alveoli themselves.

Of what significance are the spirals? We will not be in error if we assume with Curschmann that they are an indication of an exudative bronchiolitis. Their almost constant occurrence in asthma, and their peculiar form exclude the assumption that they are accidental admixtures of the sputum. Their simultaneous occurrence with the frequency and violence of the asthmatic attacks, their profuse appearance immediately after the paroxysms, and their absence in the intervals of attacks, indicate that they stand in causative relation to the attack. It may be assumed that a more or less extensive occlusion of bronchioles with these spirals causes the increasing dypsnea, but that the true paroxysm begins only with the spasm of the bronchial circular muscular fibers (Biermer) sympathetically induced by obstruction of the bronchioles and distension of the alveoli (appearing with the labored breathing). That a certain "irritable" weakness of the affected individual is also to be assumed is well known.

In 1872 von Leyden, who observed "tubularlike formations" in *isolated* cases of asthma, emphatically "refrained from expressing any opinion as to the nature of these structures," and *attributed especial significance only to the crystals.* However, the fact that these are absent in numerous cases of asthma in which the spirals are present and, therefore, cannot possibly participate in the development of the attack; and that, *on the other hand, they were invariably observed by Scheubel and others in hemoptysis parasitaria* (see page 227) *in the absence of asthma, speaks against the rôle assigned to them.*

W. Gerlach has refuted the view that the spirals are products of an exudative bronchiolitis. According to him, they can *originate only in the medium-sized and coarser bronchi* by strong twirling movements, after the manner of a waterspout or whirlwind; they *are not the cause, but the result of the asthma.* The viscidity of *scanty* secretion, the intense dyspnea, and complete perviousness of the respiratory passages are said to favor their formation. While the author readily admits that these movements may occasionally give rise to the development of spiral

mucous formations (see page 230), it appears to him more than venturesome to conclude from this possibility that the Curschmann spirals occurring in asthma are not the cause, but the result of the attack. Aside from the fact that Schmidt has demonstrated the spirals in the bronchioles at autopsy, while Gerlach admits their origin only in the medium-sized and coarser bronchi, Gerlach owes us an explanation of the following questions: Why do we invariably find the spirals here and never or but rarely in many other diseases accompanied by severe dypsnea and coughing? How can this theory be harmonized with the fact that numerous spirals can also be found in the not rarely *profuse* sputum? How can the yellow-crystal-laden spirals be explained? Why are only very *fine* spirals almost always found and large ones but very rarely?

That a spiral arrangement of mucus can *occasionally* be produced in the large bronchi by violent efforts of coughing and forced respiratory movements, has been impressed upon the author by the observation of a spiral about thirteen centimeters (!) long and from eight to ten millimeters (!) thick, which showed perfect spiral structure and a bright axis. The diameter and length at once indicated a large bronchus as the place of origin.

Asthmatic sputum is almost always characterized by its rich content of eosinophile cells. By staining dried preparations with watery solution of eosin and methylene blue or Chenzynski's solution, this fact can readily be demonstrated.

This feature can also be demonstrated in *fresh* sputum. If *1* drop of Chenzynski's solution be added to a flake of such sputum and the latter gently crushed under a cover-glass after the stain has acted for one-half minute, there will be seen after a short time—best after one hour—very numerous small and very large leucocytes with *eosinophilic granules* and *also some with basophilic granules.*

Permanent Preparations of Spirals.—They can be preserved *unstained* in glycerin or in a mixture of equal parts of glycerin and levulose. According to the author's experience, Ehrlich's hematoxylin-eosin mixture is best adapted for *staining* (see page 151). One or two drops of this solution are added to the fresh spiral, and after from five to ten minutes a cover-glass placed over it; or the mixture may be allowed to flow under the edge of the cover-glass and the excess washed out with glycerin. A ring of dammer varnish serves to fix the cover and prevents evaporation. Figs. 94 and 95 were made from photomicrographs of spirals stained in this manner.

It is not rare to find in asthmatic sputum coarse blackish and fine brownish-red *pigment-cells.* Even with the naked eye it can be surmised in what portions of the sputum these may be found. They appear as

fine, dustlike bodies imbedded in the mucoid ground substance. These cells will again be referred to when speaking of "heart-lesion" sputum.

The blood of asthmatic patients often shows a decided increase in eosinophiles during the paroxysms, especially in the severe form characterized by weeks of dyspnea accentuated by acute exacerbations.

In **pulmonary edema** the sputum is watery, chiefly serous, but slightly mucoid, greenish white, and usually intensely foamy, resembling beaten white of egg. *Microscopically* it is recognized as a *poorly cellular,* thin, mucoid secretion. It is usually observed only in dying subjects. It is repeatedly met with, however, in many chronic renal and heart patients without the attack in question ending fatally. The sputum, which is not infrequently observed in favorably progressing cases of pleural paracentesis and described as *"expectoration albumineuse,"* possesses such a strong resemblance to that just described that a distinction cannot be made. In both cases the sputum is *highly albuminous,* as the boiling test proves, and also contains some mucus, as is shown by the opalescence produced by addition of acetic acid. It is a positive indication of the transudation occurring in the alveoli and bronchioles, and in terminal cases even into the larger air-passages of the lung, which usually follows exhaustion of the left side of the heart. In *expectoration albumineuse* it is perhaps due to abnormal perviousness of the vessel-walls in the previously compressed lung.

The sputum periodically expectorated in *whooping-cough* is especially transparent and mucoid (foamy); it is usually thin, tough, and adhesive in consistency, and often unusually voluminous; so that at the end of the paroxysm mouthfuls are expelled. Not infrequently it is mixed with vomited matter.

Closer examination shows that it is a sputum very poor in cells—occasionally containing ciliated epithelium!—intensely mucoid (acetic acid reaction),—the genuine *sputum crudum* of the ancients. Only in the third stage does the secretion become less profuse and more yellow, and shows microscopically the *character* of sputum coctum which has already often been discussed.

Whether the bacteria found in the sputum by Czaplewski, Hensel, and Koplik are the exciters of whooping-cough is still undecided. They are small oval bodies whose ends usually stain more deeply (polar staining, polar bacteria) than their middle portions, whereby the form of a diplococcus is presented. In reality, they are short rods which grow in the form of delicate, droplike colonies upon the ordinary nutrient media, and best upon Löffler's serum-plates.

In **la grippe**, also, there is at first observed, but often only transitorily, a sputum crudum which soon passes over into a sputum coctum. In addition to a few mixed, muco-purulent masses, it contains purulent, globular clumps when the deeper portions of the bronchial tubes are involved. As has already been mentioned (page 76), it is in these purulent clumps that the short bacilli described by Pfeiffer are found. The more purulent character of the sputum in cases complicated with croupous pneumonia has also been discussed in connection with pneumonia (page 219).

To the naked as well as the aided eye the "**heart-lesion sputum**" (Plate IX, Fig. 18) presents a very characteristic appearance which, even during life, permits a positive conclusion as to the existence of *brown induration of the lung.* As is known, this alteration is principally found in those forms of chronic vitium cordis associated with more or less intense manifestations of congestion in the lesser circulation, particularly in mitral stenosis and insufficiency, but also occasionally to a marked degree in lesions of the aorta and in myocarditis.

The lungs of red induration are firm in consistency, heavier and less elastic than normal, and present a color shading into yellow, brownish, or reddish brown. On incision there are noticed at many points more or less large, red or dark-rust-colored spots, while the tissue itself presents a yellowish or more rust-colored appearance. The capillary loops of the pulmonary artery, which are usually covered with delicate epithelium and *project only very slightly* into the lumen of the alveoli, *here bulge toward the interior as intensely dilated tendril-like loops*, producing decided narrowing of the alveoli. Yellow, brownish-red, less often blackish pigment granules and red blood-corpuscle "shadows" can be seen in the connective tissue as well as in the alveoli, partly free, partly inclosed in round, oval, or spindle-form cells.

The sputum corresponds with this anatomic finding to a remarkable degree. It presents certain differences according as it is expectorated in times of comparative comfort or during manifestations of intense congestion, especially after hemorrhagic infarction. In the first instance it is usually scanty, and only two or three sputa of slight amount can be obtained for examination. There will be found in a purely mucoid, somewhat viscid, gelatinoid, clear or pale yellow, seldom brownish-tinged matrix, isolated or densely collected fine and coarse yellow or brownish-red granules. Now and then a somewhat dark, superficial, dustlike deposit is also observed. If such a yellow or reddish, mottled, mucous flake is placed under a cover-glass, there will at once be seen a

large number of cells arranged in rows or masses imbedded in a basement substance which is either more or less homogeneous or mixed with bright myelinlike drops. The cells are usually sharply defined, from one to five times the diameter of a colorless blood-cell, or rounded, oval, spindle, or polygonal form, and generally have one or several vesicle-like nuclei. The nucleus is often partly or completely obscured by fine and coarse granules, which fill the protoplasm. These granules are of the nature of myelin, very seldom more refractive than fat, but chiefly composed of *pigment*. It sometimes fills the cells as diffuse, golden-yellow pigment, but it is more frequently arranged within the cell-body in the form of fine and coarse granules, fragments, clumps, and *globules*. Not infrequently only a few coarse granules are seen in the myelinlike altered protoplasm. In addition to these characteristic cells there can be seen normal red and colorless blood-corpuscles, and not rarely fine and coarsely granular *free* pigment.

Shortly after the subsidence of a hemorrhagic infarction these pigment-cells are found in large numbers in the usually brownish-red-stained sputum. Cells containing coarsely granular brownish-red pigment are especially numerous, in addition to many red corpuscles, whose presence can be suspected from the macroscopic admixture of pure blood.

If pronounced manifestations of congestion are present, which are indicated by increased dyspnea, bronchitic râles, etc., the amount of sputum is often increased to eighty or one hundred cubic centimeters or even more. The consistency is somewhat diminished, but always quite thin and mucilaginous; so that the sputum shows a tendency to glide out as a coherent mass on inversion of the vessel containing it. Separation into three layers is also observed—an upper foamy-mucoid, a middle sero-mucoid, and a lower grayish-white, pigmented layer. Here and there can be found slightly purulent admixtures. Careful examination of such sputum will always show pigment flocculi, provided brown induration has developed.

In the majority of cases the pigment is of a yellow, yellowish-red, or brownish-red color, and is thereby differentiated from the carbon pigment found in almost every sputum. In many instances, however, there occurs in addition to the brownish-red pigment an almost *glittering, black* pigment, which is unquestionably produced in the same manner as the brownish red. It often occurs in large amount in addition to the latter, and is distinguished from the coal pigment inclosed in the cells by the much deeper and crystalline, glistening black.

For anyone who has carefully studied the pigment-cells here described and has compared them with those present in sections

of red, indurated lung-tissue, there can be no doubt that they are similar formations in every respect. For this reason E. Wagner designated them as *"heart-lesion cells."*

What is the nature of the pigment? The presumption that it is derived from the blood-coloring matter is fully justified.

According to Virchow, the pigment formation occurs by escape of the coloring matter from the red corpuscles and diffusion into the neighborhood, where it is collected into pigment granules or crystals; or the blood-corpuscles coalesce, either singly or in masses, and become transformed into pigment. In both instances the pigment may be yellow, red, or black; diffuse, granular, or crystalline. The blood may undergo pigment metamorphosis even in the capillaries. In addition to the pigment inclosed in cells or lying free in the tissues, Orth found *pigment thrombi* in the capillaries and even in the larger vessels. The blood-coloring matter may, therefore, be transformed into pigment without participation of contractile cells. On the other hand, the fact first observed by Langhans has often been confirmed, namely: that red blood-corpuscles shrink to a pigment granule only after they have been taken up by contractile cells, the pigment granule being subsequently transformed by division into a number of small granules. In contradistinction to crystalline *hematoïdin*, which is *aferrous*, the pigment granules and clumps almost always *contain iron;* consequently, at Neumann's suggestion, this pigment has been designated as *"hemosiderin."*

It has been shown that this (hemosiderin) can be formed only through the agency of living cells,—*i.e.*, living tissues,—whereas the aferrous hematoïdin is originated as the result of a chemic disintegration process which is effected without the intervention of living tissue (Neumann). Hemosiderin pigment is accumulated with especial frequency in the fixed connective-tissue cells, which, according to Neumann, may also have developed from wandering cells containing blood-corpuscles.

If the heart-lesion sputum containing pigment-cells is submitted to the *iron reaction*, it will be found that the majority of the cells assume the characteristic **Berlin-blue** *color*.

The test is conducted either with fresh or, better, with dry preparations by isolating the pigmented sputum flocculi and spreading them out with a *glass needle* (not an iron needle!).

It is very advantageous then to treat the preparation for from one-fourth to one hour with a 2-per-cent. solution of potassium ferrocyanid, to which from 1 to 3 drops of pure HCl has been added; or the dry preparation is first treated for two minutes with potassium ferrocyanid and afterward with from 1 to 3 drops of 0.5-per-cent. hydrochloric-acid-glycerin. The reaction is very distinctly shown by the blue color, which often becomes more intense during the following twenty-four hours.

In sputum preserved by the author for three-fourths of a year in a loosely covered watch-glass, and which at first contained only numer-

ous granular, pigmented cells, it was found that yellow and brownish-red needles and plates had developed. While the pigment-cells showed an intense Berlin-blue reaction, the extracellular formations remained wholly unaltered.

Not all pigment-cells (in the tissue of the lung and in the sputum), however, show the iron reaction. This is evidently dependent upon age. *Neither the freshly formed nor the old pigment responds to the iron reaction.*

On the other hand, according to Neumann and F. A. Hoffmann, *among the "soot-cells,"* which resemble the "heart-lesion" cells in form and size and in shape and position of the nucleus, *there are often some which give a distinct iron reaction.*

In view of the dispute as to the origin and significance of the "heart-lesion" cells, these facts must ever be kept in mind.

According to Sommerbrodt, Hoffmann, and others, the "heart-lesion" cells are alveolar epithelia; according to others, partly alveolar epithelia, partly wandering cells (Lenhartz). While the majority of observers are convinced of the significance of these cells in the diagnosis of brown induration, there is now and then some doubt in this direction, since they are also occasionally met with in croupous pneumonia, hemoptoic phthisis, and asthma.

The epithelial origin of the "heart-lesion" cells and of the morphologically similar "alveolar epithelia" is based chiefly upon the morphologic features. However, Virchow, Cohnheim, Neumann, and others have doubted this and have more or less strongly emphasized the insufficient basis for such a conclusion. Neumann also called attention to the perfect resemblance of "heart-lesion" cells to the "soot-cells" found in ordinary pharyngeal expectoration.

Bizzozero endeavored to overcome these difficulties by distinguishing *two forms of alveolar epithelia:* a broad, thin form with flat, oval nucleus containing a nucleolus and surrounded by a few protoplasm granules; and a second smaller, less flattened, more oval or polyhedral cell rich in granular protoplasm and containing one or two nuclei. These were said to be especially disposed to take up coal-dust, etc.; to show active proliferation in inflammatory processes; and, by virtue of the ready contractility of their protoplasm, to incorporate coal-dust, red blood-cells, and fat- and myelin- drops. In answer to the objection that such cells are also abundantly found in slight colds (and in the so-called pharyngeal sputum), Bizzozero retorts that it must not be assumed from this that they are not derived from the lungs, but rather that it is justifiable to conclude that "even mild catarrhs of the air-passages readily extend into the lungs!"

The author does not share this opinion. It appears to him unsafe to conclude that the occurrence of these two cell-forms (evidently iden-

tical with the "heart-lesion" cells and the "soot-cells") is always associated with participation of the alveoli. Such a view is contrary to the other *clinical* manifestations. On the other hand, the results obtained by experimental pathology are also opposed to Bizzozero's interpretation of these cells.

In young guinea-pigs which had been subjected for two hours to breathing of lampblack, Tschistovitsch never found any such pigment in the *epithelia;* but he did find it in the leucocytes, which even after two days assume an "epithelioid" character. Furthermore, in rabbits which had received intratracheal injections of swine erysipelas cultures and at the same time an injection of a carmin suspension into the jugular vein, he observed in the alveoli, after twenty-four hours, lymphocytes inclosing carmin and *large cells containing bacilli. The latter cells were morphologically identical with desquamated alveolar epithelia, but their leucocytic nature was proved by the fact that they also contained carmin.* Shall we here assume that the pigment was conveyed by the leucocytes to the epithelia? Is not the assumption of Tschistovitsch—that the carmin-holding leucocytes wandered into the alveoli, here incorporated the bacilli, and then assumed an epithelioid character—much more rational?

Since Ehrlich's communications in reference to *neutrophilic granulations* peculiar to leucocytes, it is to be hoped that this controversy may be settled.

At the author's suggestion Dr. Schlüter undertook to solve the problem by numerous staining experiments with Ehrlich's mixtures upon sputum and section preparations. It is certain that a portion of the pigmented cells cannot be stained a distinct violet with either the tri-acid or the neutral staining solution, and that, on the other hand, a certain number of them show decided violet granulations. The appearances obtained, however, were by no means very instructive. The best results were secured at my suggestion by staining *fresh* sputum preparations. Synchronously with our investigations, von Noorden endeavored to elucidate this question in a similar manner. He concluded that *leucocytes as well as alveolar epithelia* participate in the genesis of "heart-lesion" cells.

From what has been said, it appears to the author that the assumption most nearly corresponding to our present knowledge of the subject is that the *"heart-lesion" cells are chiefly wandering cells,* which have either taken up free pigment or have formed it from red blood-corpuscles incorporated by them; furthermore, that some "heart-lesion" cells possibly originate from alveolar epithelia which have either independently taken up pigment or have it conveyed to them through the chromatophores (Karg), as in the external skin, by the circulating fluids, or through the agency of active cells.

Are the "heart-lesion" cells of pathologic value in brown induration of the lungs? Unquestionably! Occasional contradictory observations which demonstrate the occurrence of hemosiderin cells in pneumonia, phthisis, or asthma are not to be seriously considered in view of the fact that the pigment-cells are *constantly* found in *great numbers* in chronic vitium cordis. In the former instances an occasional pigment-speck is seen or only a few pigment-cells can be demonstrated by microscopic examination; in the latter instance the mucoid sputum, which is *macroscopically characteristic,* contains numerous small and large clumps and grains of pigment and great numbers of *pigment-granule cells.*

Again, in the former instance they must be carefully sought for, and only occasional cells are found in the microscopic field; in the latter instance they cannot at all be overlooked; indeed, they are so numerous that one is often almost led to believe that a tissue preparation is under examination. Very often the cells (in the sputum as well as in the lungs) give the iron reaction; not infrequently they respond indifferently. For this reason the proposed name "hemosiderin cells" appears to be wholly inappropriate.

It has already been mentioned that the "heart-lesion" cells are especially numerous in the sputum for a time after the occurrence of a **hemorrhagic pulmonary infarction.** Apparently, especially favorable conditions for the formation of pigment are then offered, since the process usually occurs in patients with heart disease (mitral stenosis) and brown induration, and consists of the diffuse infiltration of the interstitial tissues, alveoli, and bronchioles with red blood-corpuscles corresponding to the area of the infarcted portion of the lung.

The *sputum in fresh infarction* sometimes consists of pure, somewhat dark blood; more frequently it is mixed with mucus, and less so with air. The bloody expectoration continues for several days or subsides after a few hours, depending upon the extent of the infarct. Microscopically there are found unaltered, red blood-corpuscles, often arranged in rouleaux, and usually isolated pigment-cells which increase in number with diminution of the purely bloody constituents.

In **hysteria** a peculiar sputum is occasionally observed, which

is usually easily coughed up, and owing to its distinctly *bloody* character may often give rise to a suspicion of phthisis. The sputum may *for days* resemble thin raspberry-jelly. It generally presents *for weeks a uniform reddish, fluid or brothlike character,* and deposits numerous minute, gray granules. Delicate streaks of pus may or may not be present. The quantity varies between twenty-five and one hundred cubic centimeters. It is chiefly expectorated at night or early in the morning. Physical signs on the part of the respiratory tract are absent. The general appearance and other manifestations are indicative of hysteria.

Microscopic examination does not generally reveal as many red blood-cells as would be expected from the color, but *great numbers of squamous epithelia,* leucocytes, and micro-organisms are present. Wagner once observed bodies resembling *trichomonas.*

That a certain amount of caution should always be exercised in the interpretation of such bloody sputum is shown by the observations of Wagner, in one of whose patients tubercle bacilli were subsequently found in the sputum. In this regard it should be remembered that patients who for years have suffered from hysteria not infrequently succumb to tuberculosis. *The continuance for weeks of a sputum of a bloody-mucoid character, the great number of squamous epithelial cells* (from ten to twenty were found by the author in a field of from 250 to 350 magnification!), *and the absence of tubercle bacilli are indicative of hysteric sputum.*

It is most probable that the peculiar expectoration is derived from the oral cavity, and is induced by suction.

The diagnosis of the **neoplasms (tumors)** occurring in the lungs may often be greatly facilitated by inspection of the sputum. It is generally scanty and almost without exception muco-hemorrhagic, but so thoroughly incorporated that a rose or flesh-water color or a *raspberry-jelly*-like character is apparent. *Olive-green* or saffron-yellow colored sputum is occasionally observed.

At times pure blood may also be expectorated at intervals in scant or large amounts for days, weeks, or even months. A severe hemoptysis is not rare, but fatal hemorrhage occurs only

in exceptional cases. Very rare, also, are the cases in which *tumor-fragments* have been observed in the sputum (Ehrlich, A. Fränkel) and in which "multiformed cells, which were arranged in large clumps, and occasionally showed concentric lamellations and large swollen nuclei," were present.

Such findings, however, are rare, and it is therefore advisable to seek for other signs. According to the author's experience, which is based on a dozen such cases observed at necropsy, the occurrence of numerous *fat-granule spherules (Fettkörnchenkugeln)* are of especial diagnostic value. They are distinguished

Fig. 97.—Fat-granule Spherules in Pulmonary Carcinoma. × 350.

from myelin-cells by their size and the highly refractive brilliancy of the individual granules composing the masses. They are very probably derived from fatty-metamorphosed epithelia (carcinoma-cells) (Fig. 97).

In addition to the fat-granule spherules, peculiarly formed epithelia, which otherwise are not met with in the sputum, are of interest. They are frequently encountered in carcinoma of the lung and are not rarely present in large shreds. Hampeln has drawn attention to the fact that they are always free of pigment. The author does not attribute as much significance to them as to the fat-granule spherules.

IV. EXAMINATION OF THE SECRETIONS OF THE MOUTH AND OF THE GASTRIC AND INTESTINAL CONTENTS.

In affections of the digestive apparatus many elements may be found in the discharges *per os* or *per anum*. Their proper interpretation requires a knowledge of anatomic relations, and especially of the mucosa of the entire intestinal canal. Therefore a brief *résumé* of the normal anatomy will be presented.

1. BRIEF ANATOMIC DESCRIPTION.

The *mucous membrane of the mouth* consists of lamellated squamous epithelium covering the membrana propria. The latter is provided with numerous large and small papillæ, and is composed of elastic fibers and delicate bundles of connective tissue. Within the propria numerous tubular mucous glands are found, some of which are decidedly branched. The excretory ducts of these glands are likewise provided with pavement epithelium.

The *tongue* is covered chiefly with thickly lamellated pavement epithelium. The same variety of epithelium also covers the papillæ filiformes, fungiformes, and circumvallatæ, and in some places is heaped in many layers. Hornified epithelium occurs only upon the filiform papillæ, and is characterized by absence of the nucleus in the individual squamæ. At the root of the tongue, in the region of the so-called lingual crypts, adenoid tissue is found between the papillæ circumvallatæ and the epiglottis. At this point numerous leucocytes constantly wander from the adenoid tissue of the propria and mingle with the oral mucus as the so-called *salivary corpuscles*.

The pharynx possesses a multilamellated pavement epithelium which extends over the numerous papillæ and mucous glands. The mucous membrane of the naso-pharyngeal cavity is provided with multilaminated, ciliated cylindric epithelium. A large amount of adenoid tissue is present upon the pharyngeal tonsils and especially upon the true tonsils. This tissue also produces numerous salivary corpuscles.

The laminated pavement epithelium of the pharynx is continuous throughout the esophagus. The mucosa of the esophagus is provided with papillæ and is surrounded by the submucosa, which contains many mucous glands, and by dense muscular and fibrous coats.

The *mucous membrane of the stomach* is composed of epithelia, membrana propria, muscular layer, and submucosa. The cells composing the epithelium are of a mucus-forming, cylindric variety. The membrana

propria contains closely arranged glands which are designated as *fundus* (peptic) and *pyloric glands.* They are differentiated from each other by the fact that the pyloric glands contain only cylindric cells resembling the so-called *chief* [or *central*] cells (Ebstein), while the fundus glands contain *parietal* [or *acid*] *cells,* in addition to central cells. The chief [or central] cells are of a low cylindric type and contain a sharply defined nucleus imbedded in a granular protoplasm. The parietal [or acid] cells, *which are much enlarged during digestion,* appear in a more rounded form. The chief cells are greatly clouded and somewhat swollen during the height of digestion. Thus, the distinct difference noted in the fasting stomach is somewhat obscured. Both groups of glands have a tubular structure.

The *intestinal mucosa* is covered by cylindric epithelium, interrupted here and there by (mucoid) oval goblet-cells.

2. EXAMINATION OF THE ORAL CAVITY.

Reference has repeatedly been made to the frequent occurrence of *cocci, bacilli, spirilla, leptothrix,* and like vegetations in the mouth. Usually their presence may be considered physiologic. They are regarded as pathologic only when they occur in excessive numbers, such as are observed in continued neglect of the toilet of the mouth and when numerous hollow, decayed teeth are present.

The **soor** [thrush, aphtha, or sprue fungus: *oïdium albicans* —Brooks] fungus is deserving of more attention (see page 90).

This fungus is usually found in children and debilitated adults. It begins upon the mucosa of the soft palate, tongue, or cheeks. Extensive deposits covering the oral and pharyngeal cavity form by coalescence of a number of individual fungous eruptions. The characteristics of this fungus are the pure white or dirty-grayish-yellow color, and the readiness with which the deposit can be dislodged without injury to the mucosa. If a small portion of the "pseudomembrane" is placed under the microscope, a diagnosis can be readily made.

Accompanying the soor fungus in infants, but also without it, symmetric, whitish or whitish-yellow areas are not infrequently seen in the region of the hamuli pterygoidei. They are called *Bednar's aphthæ.* The surface bleeds readily when the round or oval foci (from two or four to eight millimeters in diameter) are disturbed. Erosions may occur, and if material is scraped from such areas, dried, and stained, staphylococci and streptococci exclusively will be found.

The **gonococcus** rarely invades the mucous membrane of the mouth in the newborn. This condition has already been described (page 44).

Innumerable bacteria common to the oral cavity, in addition to pus-corpuscles and fatty *detritus,* are found on microscopic examination in the yellow or whitish-yellow plugs of **acute angina tonsillaris.** A specific species has not been discovered.

Yellowish-gray, offensive *plugs,* which can be crushed flat beneath a cover-glass, can often be removed from the tonsils of those who suffer with frequent attacks of lacunar inflammation. Microscopically they show, in addition to countless bacteria, fatty *detritus* and fat-needles.

Similar bodies, occasionally incrusted with lime, are expectorated by many persons during either coughing or hawking. They may be free or imbedded in mucus, and often give rise to much discomfort. These bodies are formed either in the lacunæ of the tonsils or in the mucous follicles of the lateral walls of the pharynx. *These plugs are usually about the size of a milletseed, but may be found as large as a bean!* The largest observed by the author were the size of a small pea.

Cyst-formations in the tonsils are occasionally seen in such patients. They vary from the size of a pea to that of a cherry. The fluid evacuated by superficial puncture is watery or paplike in consistency, and reddish or yellowish red in color. In addition to fatty *detritus* and fat-needles, the author repeatedly found in such cysts *cholesterin plates* and *once hematoïdin plates and needles.* In addition to cholesterin, he has seen large, slightly glistening bodies which, to a certain degree, resembled a large egg with yelk. *They disappeared upon repeated addition of ether;* the transitional stages often presented strong resemblance to a transverse section of a large seashell.

In **tonsillar** and **retropharyngeal abscess,** numerous pus-cells in a more or less advanced stage of fatty metamorphosis, *much free fat, and large numbers of bacteria* are found in the yellowish-white purulent matter, which is usually quite thick in consistency. Small pigment granules and flakes are not infrequently observed.

In one case, which has already been mentioned on page 95, the author observed masses of *leptothrix* associated with numerous *cercomonas forms* (see Fig. 47). Numerous eosinophiles were also present.

In view of the great importance of the tonsils as a port of entry for infectious bacteria, it is advisable to devote greater attention to the bacteriologic examination of such plugs. The experience of Birch-Hirschfeld is sufficient proof of this, since he was twice able to find tubercle bacilli in such masses. Carious teeth are quite as worthy of attention as the tonsillar lacunæ.

The diagnosis of **croupous** and **diphtheritic affections** of the fauces is greatly facilitated by microscopic examination, especially in the early stages of the disease.

A more or less dense fibrous reticulum (see page 207, Fig. 88) is found in the white deposits of the later stages. Owing to the difficulty in teasing it, the construction of this reticulum can be made out only at the periphery of the fragment. On addition of acetic acid (1 to 2 per cent.) the round cells and epithelia, with their nuclei, are distinctly outlined imbedded in the clear reticulum. If the diphtheritic process extends to the respiratory passages, long croupous coagula, which have already been described (page 200), may be expectorated.

Tuberculosis of the oral and pharyngeal cavities is very seldom observed.

As a rule, it first appears in the form of partly isolated, partly conglomerate, miliary nodules, which occasionally occupy the margins of the tongue. It manifests greater preference, however, for the lateral and posterior walls of the pharynx. The invariable tendency to disintegration usually leads to superficial, irregularly outlined, and often eroded ulceration. The base is gray or of baconlike color. Grayish-white, transparent nodules at the periphery of the ulcers are important in diagnosis.

The diagnosis, however, is established only by demonstration of tubercle bacilli.

Some of the secretion is scraped from the caseous base or the margins of the ulcer, then triturated in a watch-glass, with addition of a few drops of physiologic salt solution if necessary, and cover-glass or slide preparations made and stained in the manner already described (page 49).

The specific bacillus can often be demonstrated by this method. If this method is unsuccessful, portions of the tissue should be removed, hardened in alcohol, and stained in sections.

3. FINDINGS IN DISEASES OF THE STOMACH.

Microscopic examination is seldom of decisive value in the diagnosis of disturbances of the stomach. The gastric contents obtained either by vomiting or mechanical means (lavage, bucket, etc.) is used for this purpose. In addition to coarser food-remnants, foreign bodies, and abnormal discolorations due to admixture of blood and bile—all of which are perceptible to the naked eye—there are found:—

(a) Food-remnants of animal and vegetable diet. More or less completely disintegrated *muscle-fibers*, in which the transverse markings are usually well defined, though often somewhat indistinct. *Remnants of milk* in the form of casein flocculi, fat-droplets, etc.; *vegetable cells* in great variety, which give a distinct starch reaction.

(b) Mucus, which is recognized with certainty by precipitation with acetic acid. It is secreted partly by the mucosa of the stomach and partly by that of the esophagus and pharynx.

(c) Blood occurs in large amounts in gastric hemorrhage. At first it is usually mixed with remnants of food, but in continued hemorrhage it appears pure. It is sometimes bright red, but more often dark red. When examined microscopically, it shows red blood-corpuscles somewhat shrunken or partially decolored. If it appears in a dark-brown, coffee-ground-like form, the blood-corpuscles can usually no longer be recognized. On the other hand, the blood-coloring matter can be demonstrated by chemic and microscopic examination (see "Blood," pages 189 *et seq.*).

(d) Epithelia and *tubular ducts* are very rarely observed in vomited gastric contents, but they are occasionally seen in the material secured by artificial means (siphon, etc.). The epithelia are in distinct cylindric forms or they are "chief," or parietal, cells derived from the tubular glands (Fig. 98).

Very rarely the specific cells of neoplasms are encountered. These will subsequently be discussed (see "Carcinoma").

(e) **Parasites.** — 1. Vegetable parasites are constantly present. Cocci, bacilli, and spirilla are found in all gastric contents, even the most normal. *Sarcina ventriculi* and *yeasts* are commonly found in stagnation, whether free HCl is present or not. They are readily demonstrated by taking some of the gastric contents from the bottom of the vessel with a pipette and examining a fresh drop (with or without dilution with water) under the microscope. The *sarcina ventriculi* (Fig. 98, *i*) is distinguished by its distinct tetrad form and packetlike arrangement. If once seen it is impossible to mistake it. The individual cells are more or less finely granular. Oppler has recently distinguished by cultivation five species of sarcina, of which the *orange-yellow (aurantia)*

sarcina is characterized by the fact that it grows luxuriantly on an acid nutrient medium, while the other varieties grow only upon alkaline media. Cultivation is most successful in 2-per-cent. glucose-gelatin.

The *yeast fungus* (Fig. 98, *k*) has already been considered (page 85).

In *cholera* the characteristic bacilli may occur in the vomit, particularly in the mucous flocculi.

2. Animal parasites are seldom observed in vomited matter. When present, they are usually small or large roundworms (*ascaris lumbricoides*, page 113), though oxyuris and anchylostomum, as well as hundreds of living larvæ of the ordinary housefly, are occasionally found.

Fig. 98.—Gastric Contents. Collective Microscopic Picture. × 350.

a, Air-bubble. *b*, Oil-droplet. *c*, Muscle-fiber, nearly digested. *d*, Potato starch. *e*, Swollen rye starch. *f*, Leguminous starch. *g*, Various vegetable cells. *h*, Vegetable hair. *i*, Sarcina. *k*, Yeast fungi. *l*, Gastric gland-cells.

(f) Pus may be mixed with vomited matter in cases of rupture of oral and pharyngeal abscesses or in affections of the respiratory tract. Pus comes from the stomach itself only in rare instances, as in cases of phlegmonous gastritis after burning and corrosion, or in perforation of pus-foci in the region of the stomach. [According to most authorities, the constant occurrence of pus in fetid or nonfetid stomach contents is diagnostic of carcinoma. Occasionally, however, as the author states, pus may be present when a perigastric abscess ruptures into the stomach, but in such instances mucus is usually absent.—Brooks.]

(g) More or less accidental admixtures may be found, such as small and large foreign bodies; small stones, hair, poppyseeds, henbane-seeds, etc. They are raised by either spontaneous or induced vomiting.

(A) APPEARANCE AND MICROSCOPIC CHARACTERS OF VOMITED (OR SIPHONED) GASTRIC CONTENTS IN CERTAIN DISEASES.

1. In acute and chronic **gastric catarrh** the amount of mucus is markedly increased and the number of bacteria is usually augmented; in the chronic form yeasts and sarcina are often found; round cells are common; epithelia are rare. In siphoned material semilentil-sized pieces of superficial mucosa are occasionally observed. Microscopically it shows a very beautiful continuous layer of epithelium which appears to be pierced by several glandular mouths. Such layers are torn off by mechanical violence in raising the stomach contents and occur more often in gastritis chronica. By suitable care these injuries can be avoided.

2. In **ectasis** the pathologic manifestations are increased. Sarcina and yeasts occur in large numbers, especially when the dilatation is *not* caused by carcinoma. In the presence of malignant disease these fungi are not so constant. On standing for several hours distinct fermentation and a "rising" of the vomited matter occur.

3. **Ulcer rotundum** frequently gives rise to *bloody* vomit. In addition to blood mixed with remains of food, pure blood may be expelled to the amount of even one liter or more. It is rarely bright red, but usually dark red, fluid, and coagulated in clumps. Occasionally it appears in the form of brown, coffee-ground, or tealike masses. *Microscopically* partially crenated red blood-corpuscles are usually found. If they are totally destroyed, the chemic or spectroscopic demonstration of blood-coloring matter should be made. The vomited matter is usually acid in reaction.

4. In **carcinoma of the esophagus and stomach** portions of the neoplasm may occasionally be torn off and brought up in the eye of the stomach-tube during sounding, and the diagnosis thus established. Specific formed elements are very rarely found in the *vomited material.* It is very difficult to decide in a given case whether the morphologic elements are really derived from a neoplasm or the normal mucosa. *There is no specific carcinoma-cell.* Only truly "concentrically lamellated" cell-groups, or *"cancer-pearls,"* can be regarded as of positive significance.

Isolated epithelial shreds which microscopically show no evidence of an alveolar structure are wholly without significance.

The author has rarely found the significant "cancer-pearls" in cases of carcinoma of the esophagus or stomach.

The amount and character of the masses vomited in carcinoma of the stomach depend upon the location of the neoplasm. Carcinomata located in the *cardia* or adjacent parts generally cause early vomiting of the ingested food. It is expelled but slightly altered and imbedded in mucus. It possesses a stale odor, and, in sloughing carcinoma, an extremely offensive odor.

The fact that in cancer of the stomach the siphoned gastric contents diffuses a *repulsive odor* is of great diagnostic value. Obscure cases in which eructation, vomiting, and palpable tumor are absent can be correctly interpreted by this odor, as I have found in several cases which presented irregular chronic fever without prominent gastric symptoms.

The vomitus in **pyloric carcinoma** is usually very profuse and has an offensive acid odor. It apears gray or dark brownish, and often contains large amounts of food in a state of greater or less transformation. The color of the vomited material ranges from coffee- to chocolate- colored, according to the amount of the hemorrhage. On standing, stratification occurs, the heavier food-remnants settling to the bottom. A watery, dirty, cloudy, muco-foamy layer forms the top. A "doughy rising" is often observed, similar to that which occurs in ectasis.

Microscopically sarcina and yeasts are often found with food-remnants and numerous bacteria. Red blood-cells are rarely present. The demonstration of blood-coloring matter can usually be done by chemic or spectroscopic methods.

5. **Acute phlegmonous gastritis** always causes vomiting. Pus is not necessarily present in the vomited material. Epithelial shreds, however, are always observed. Leube found pus, although only a violent gastritis, with unusually intense purulent secretion upon the free surface of the mucosa—*without participation of the submucosa*—was present.

(B) TESTING OF GASTRIC FUNCTIONS.

I. Testing Gastric Secretion by Chemic Examination of the Stomach Contents.

As the vomitus is usually altered by a large amount of mucus secreted by the mucosa of the esophagus, pharynx, and oral cavity, the gastric contents secured with the stomach-tube

is almost exclusively employed for examination. The odor and macroscopic features of the expressed contents obtained one hour after a tea breakfast[1] or four hours after Leube's test-meal[2] are first observed. The contents are then filtered and subjected to chemic examination. The reaction (with litmus-paper) and the presence of free HCl are first determined. Then the quantitative determination of HCl and the qualitative examination for lactic acid are undertaken; and, finally, the determination of pepsin and milk-curdling (lab) ferment and the degree of proteid and starch digestion.

With very rare exceptions, the gastric contents thus obtained have an acid reaction. This is chiefly due to free and combined HCl (with bases and proteid bodies); and also to organic acids, of which lactic and less often butyric and acetic acids are of chief importance. These also may occur in a free or combined state. Finally the acid phosphate salts participate in the acid reaction in no small degree.

Significance and Demonstration of Free HCl.

It is certain that the function of free HCl, as was first emphasized from a chemico-physiologic standpoint, rests chiefly in its *antiseptic* action. Its peptonizing influence is of secondary importance. The amount of HCl occurring in the stomach suffices to kill the majority of *putrefactive micro-organisms* and a number of infectious bacteria introduced with the food. As the rhythmic peristaltic movements of the stomach constantly bring new portions of the ingesta into immediate contact with the glandular surface, the antiseptic influence of the strong mineral acid constantly secreted can be fully exerted upon the bacteria present.

The peptonizing action of the gastric juice is certainly of no small importance, even though it can be wholly supplanted by the

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[1] Two cups of black tea without sugar or milk and one dry roll.

[2] One soupplateful of gruel-soup, one-third pound boiled beef, one roll, and water. [**Ewald-Boas** test-meal: One roll without butter, or two slices of stale homemade bread, and one and one-half cupfuls or glasses of water. Remove contents of stomach after one hour. The quantity usually obtained varies from 50 to 150 cubic centimeters. Free HCl appears in from thirty to thirty-five minutes after ingestion. The stomach should be emptied in from two to two and one-half hours after the meal. Total acidity is about 55 to 60; free HCl, 20 to 30.—Brooks.]

functionating pancreas. On the other hand, the organism does not possess a substitute for the antiseptic properties of the acid. The mucosa of the pars pylorica secretes pepsin only, and that of the fundus pepsin and HCl. Under normal conditions the gastric juice may contain 0.15 to 0.3 per cent. of free HCl.

Aside from physiologic experience, Kast has shown that the more or less intense proteid putrefaction in the intestine depends inversely upon the HCl disinfection of the food in the stomach. He neutralized the gastric HCl by the administration of large doses of bicarbonate of soda and always observed a parallel increase of the ethereal sulphates in the urine (page 292). Mester also demonstrated this fact in dogs. He rendered them absolutely "chlorin-free" by feeding with dechlorinated meat. As soon as they were fed with putrid chlorin-free meat intense intestinal putrefaction at once began. On the other hand, when the animals, in consequence of restoration of chlorin ingestion, were able to elaborate free HCl, the amount of ethereal sulphates at once declined in spite of further feeding with decomposed meat.

(a) Qualitative Demonstration of Free HCl.

1. Congo paper is turned blue by *free acids*. Free HCl will alone cause a distinct cornflower-blue color. Lactic acid produces the latter shade of color only in a concentration which never occurs in the stomach. As a rule, this test can be performed by simply dipping a strip of Congo paper in the chyme. Admixture of mucus and fat may occasionally interfere with the test and necessitate filtration. The reaction is then somewhat weakened.

2. Methyl-violet solution is changed to a *sky-blue* color by traces of free HCl.

A weak, but distinctly blue, watery solution is prepared. It is divided into equal parts, placed in two test-tubes, and a few drops of the filtrate is added to one of the tubes. In the presence of free HCl a sky-blue color is produced which is strongly marked on comparison with the control solution.

3. Tropæolin.—The *yellowish-brown alcoholic* solution of tropæolin changes to *ruby-red* on addition of dilute HCl (lactic and acetic acids). According to Boas, this substance can be employed in the following manner as a certain test for HCl:—

Three or 4 drops of concentrated alcoholic solution of tropæolin and an equal amount of the chyme filtrate are mixed together in a porce-

lain dish. On heating gently, when free HCl is present, distinct lilac or blue streaks will be seen at the margin of the fluid. These streaks are *never* produced in a similar manner by organic acids.

4. Günzburg's test with phloroglucin-vanillin:—

Three or 4 drops of this reagent—composed of 2 parts of phloroglucin, 1 part of vanillin, and 30 parts of alcohol—are added to the same number of drops of the filtrate in a porcelain dish. On heating over a small flame (alcohol or Bunsen), carefully moving the fluid back and forth in the dish, the presence of free HCl is shown by the development of a *brilliant-red color*. Care should be taken not to bring the fluid to a boil, as the reaction does not take place at boiling heat.

The test can also be made with filter-paper, prepared with Günzburg's solution. A strip of such paper which has been dipped in the stomach contents turns a bright red on heating.

This test can always be relied upon to demonstrate the presence of free HCl, because the reaction is *never* produced by organic acids. Unfortunately Günzburg's reagent is not permanent. It often assumes a deep-brownish-red color, and is then useless for testing. It is advisable, therefore, to keep separately alcoholic solutions of vanillin and phloroglucin, and, when it is necessary to make a test, add 1 or 2 drops of each solution to the dish containing the gastric contents.

After many years of experience the author has found that Congo-red paper is best adapted for quickly demonstrating the presence of free HCl, and Günzburg's solution for more positive demonstration.

According to Ewald, Congo red is turned blue by 0.1 per mille, the watery solution of methyl-violet is turned sky-blue by 0.024 per mille, tropæolin solution is turned brown by 0.25 per mille, and Günzburg's reagent shows the presence of even 0.05 of HCl per mille.

As a rule, the **qualitative** *determination of free HCl suffices for practical purposes.* This is especially true in regard to therapeutic treatment in those cases in which *complete absence of free HCl has been demonstrated.* In the experience of the author, such instances are by no means rare. In this respect, the fact that free HCl *is wholly absent* in nearly all cases of gastric carcinoma (at least 90 per cent.) is most important. An exception is found only in those cases in which the carcinoma has

developed upon the base of *gastric ulcer*. In such instances as much as 2.8 per mille of free HCl can be observed, as the author has been able to confirm by necropsy investigation.

Free HCl is absent in that rare disease *atrophy* of the gastric mucosa. It is also absent in many cases of *acute dyspepsia* and in *many febrile affections,* as well as in at least half of the cases of *chlorosis* and in a large number of *chronic dyspepsias* (alcoholism). It is *nearly always present* in ulcer of the stomach, sometimes in large amount (up to 6 per mille), and not infrequently continues in this condition even when a partial transformation into carcinoma has occurred. The nervous dyspepsias present the most marked deviations: hyperacidity and hypersecretion, as well as diminution of free HCl can be observed, and not rarely both conditions are found at different times in the same individual.

In the *empty* (fasting) stomach of healthy subjects moderate amounts of HCl are repeatedly found. Only the occurrence of large amounts and high HCl percentages (0.5 per mille and over) is of pathologic significance (continuous gastric flow).

(b) Quantitative Estimation of HCl.

It is of chief importance to decide whether only the free, physiologically active HCl or that combined with bases is to be estimated. The literature of the last decade is replete with more or less valuable methods for quantitative estimation. A *résumé* of the different procedures cannot be given in this place. A critical analysis can be found in the admirable treatise of Martius and Lüttke.[1] [Ewald and Boas also give in their text-books most of the methods in detail.—Brooks.]

The following method will be found sufficient for practical purposes:—

1. Estimation of the Total Acidity.

In this procedure both the physiologic free and combined HCl are determined. Furthermore, all the remaining *organic* (free and combined) acids and the acid salts (especially phosphoric acid) are included. Lactic, butyric, and acetic acids are

[1] F. Martius and J. Lüttke: "Die Magensäure des Menschens," page 193. Stuttgart, Enke.

the chief organic acids. The reaction of these will be discussed later. If their presence can be excluded with any degree of certainty, the estimated total acidity can, in practice, be considered as the *expression of the total amount of HCl secreted.* A high degree of acidity, even in cases in which the absence of free HCl is recognized by the color-reactions already mentioned, will indicate a relatively favorable condition, because it may be assumed that active action upon the bacteria and a thorough saturation of the proteid substances occur. The nature of the food ingested must be taken into account in making this estimation in so far as the secreted HCl is seized upon—for example, by *milk*—to a marked degree, especially by the phosphoric acid salts and the casein. In this way is explained the fact that free HCl is seldom found in healthy suckling infants. Low percentages of the total acidity always indicate insufficient glandular function.

Method of Determination.—The filtrate of the gastric contents is titrated with one-tenth normal[1] sodium hydrate, using phenolphthalein (or litmus tincture) as an indicator. Decinormal sodium hydrate solution is allowed to flow, drop by drop, from a burette into a porcelain dish or beaker glass containing from 5 to 10 cubic centimeters of the filtrate. To this 1 or 2 drops of a 1-per-cent. alcoholic solution of phenolphthalein is added. The reagent in the burette is added, 1 drop at a time, to the gastric juice, and constantly stirred, until a distinct and permanent red color appears. The total acidity is expressed, for the sake of simplicity, in percentages of normal sodium hydrate. For example, if 8 cubic centimeters of normal sodium hydrate were used for 10 cubic centimeters of the filtrate, we speak of 80-per-cent. total acidity. Or we estimate the acidity direct as to HCl upon the basis that 1 cubic centimeter of normal sodium hydrate corresponds to 0.00364 of HCl. In the above instance, therefore, we can reckon the acidity equals eight times 0.00364 or 0.29 per cent. HCl.

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[1] By normal solution is meant a fluid which contains in one liter as many grams of a body as equals its molecular weight. The molecular weight of chlorin, for example, is 35.5; that of hydrogen, 1; that of the union of HCl, $35.5 + 1 = 36.5$: *i.e.*, a normal solution of HCl contains 36.5 grams of chemically pure anhydrous HCl per liter. In the same manner is estimated the contents of a normal potassium solution from the data: K equals 39; H equals 1; O equals 16; total, 56 grams.

Since the union of caustic potash and HCl to form a neutral salt occurs in the proportion of 56 to 36.5, according to the formula $KHO + HCl = KCl + H_2O$, it is plain that similar volumes of normal potassium and hydrochloric acid solutions must exactly neutralize each other. The preparation of such solutions consumes much time. Normal hydrochloric acid and potassium hydrate are officinal; the decinormal solutions necessary for medicinal purposes are prepared with sufficient accuracy by addition of 1 part of normal solution to 9 parts of water. For more detailed information, see text-books on volumetric analysis, especially Medicus, "Maassanalyse für Mediciner" (Stuttgart, 1888).

2. Quantitative Estimation of Free HCl According to Method of Morner and Boas.

In this test a watery solution of Congo-red, which we have already learned is a very serviceable reagent for demonstration of free HCl, is added to the filtrate. In the presence of free hydrochloric acid the red color is changed to blue. The mixture is now titrated with decinormal sodium hydrate solution until the Congo-red color is permanently restored. The number of cubic centimeters of sodium hydrate solution used corresponds to the amount of free HCl.

Boas has very properly drawn attention to the fact that the presence of organic acids does not usually interfere with the result. In cases in which Uffelmann's reagent or the odor indicates a considerable amount of organic acids, however, it is necessary to remove this source of error by repeatedly shaking with ether before estimating the HCl.

3. The Author's Method.

The following method appears to the author to be the most serviceable: To 10 cubic centimeters of the filtered gastric juice is added from a burette decinormal sodium hydrate solution, drop by drop. After each addition of from 5 to 10 drops, a drop of the mixture is taken with a glass rod and allowed to fall into a dish containing *dilute* (about 2-per-cent.) solution of Congo-red. As long as the HCl of the gastric juice is not neutralized, the drop produces a deep-blue color in the reagent. Sufficient sodium hydrate is, therefore, added until the drop no longer causes the development of a blue ring. When this point has been reached, the number of cubic centimeters of decinormal sodium hydrate used is multiplied by 0.365. The number thus obtained gives the percentage of hydrochloric acid. This method occasionally fails in the presence of organic acids.

In order to determine the *total acidity* the fluid previously titrated for free acid is treated with 2 or 3 drops of a 1-per-cent. *alcoholic solution* of *phenolphthalein,* and decinormal sodium hydrate added, drop by drop, under constant stirring with a glass rod, until the red color becomes permanent.

4. Quintard's Method.

[The following simple method is employed by Prof. Edward Quintard in his clinic at the New York Post-graduate Medical School:—

The volumetric analysis of a compound is the determination of the quantity of a standard solution required to satisfy a reaction in a known quantity of the compound.

In volumetric analysis of the stomach contents, certain standard solutions are used. A definite quantity of the gastric contents or filtrate is titrated after a so-called "indicator" has been added. The "indicator" is a substance which shows by change of color or some other visible effect the exact point at which a given reaction is complete.

The standard solution most frequently used in the titration of the gastric contents is decinormal sodium hydrate. The "indicator" varies according to the quality of the substance to be determined.

In order to obtain a normal solution of any particular substance it is first necessary to learn its molecular weight. This having been determined, an equivalent amount of the substance in grams is dissolved in 1000 cubic centimeters of distilled water. A decinormal solution is one-tenth as strong as the standard normal solution. For example, to make a normal solution of oxalic acid we find that the molecular weight of oxalic acid ($H_2C_2O_2 + H_2O_2$) is 126; but as it is dibasic, the normal solution would contain one-half of this (63 grams), which is the number of grams to be dissolved in 1000 cubic centimeters of water at 15° C. to make a normal solution. A decinormal solution of oxalic acid is made by dissolving one-tenth of 63 grams—*i.e.*, 6.3 grams—in 1000 cubic centimeters of distilled water. As dilute oxalic acid deteriorates very rapidly, a fresh solution should always be employed.

From what has been said, it would seem to be a very simple matter to make a normal solution. In fact, however, this is not so easy. This is due to the fact that absolutely pure chemicals are hard to obtain. This is particularly true as regards sodium hydrate (NaOH), which, owing to its hygroscopic properties, always contains a certain amount of water.

A good way to make a decinormal sodium hydrate solution is as follows: Take chemically pure sodium hydrate (Merck's), made from pure sodium. Chemically pure oxalic acid is taken for control test. Thoroughly reliable oxalic acid is sold by Merck under the name of "Acid Oxalic, Highest Purity." The molecular weight of sodium hydrate is 40. Remembering the rule to be followed in making up a normal solution, weigh out 40 grams of it and dissolve in 1000 cubic centimeters of distilled water. In order to make a decinormal solution, take one-tenth of 40 grams (4 grams) and dissolve in 1000 cubic centimeters of distilled water. As has already been stated, sodium hydrate is hygroscopic and always contains a certain amount of water, which must be considered in making a decinormal solution. This is obviated by dissolving 4 grams of sodium hydrate in about 950 cubic centimeters of distilled water. One decigram of pure oxalic acid is then weighed out and dissolved in 10 cubic centimeters of distilled water. Then add $^1/_2$ drop of a 1-per-cent. alcoholic solution of phenolphthalein as an indicator. Phenolphthalein is a substance which shows no color in acid solutions, but which gives a red reaction in alkaline solutions. Now titrate the oxalic acid solution with the sodium hydrate solution. One decigram of oxalic acid is exactly neutralized by 15.9 cubic centimeters of

decinormal sodium hydrate solution. Neutralization occurs at the instant the phenolphthalein indicator shows red. Now, as less than 1000 cubic centimeters of distilled water were used to dissolve 4 grams of sodium, it is very probable that the red color will appear too early in the oxalic acid solution; that is to say, before quite 15 cubic centimeters of sodium hydrate solution have been added. If this is the case, add a little distilled water (about 10 cubic centimeters) to the sodium hydrate solution and titrate a fresh decigram of oxalic acid solution. Repeat this process until exactly 15.9 cubic centimeters of sodium hydrate solution added to 1 decigram of oxalic acid in solution causes the phenolphthalein indicator to turn red. When this occurs the decinormal solution is ready for use.—Brooks.]

By multiplying the total amount of decinormal sodium hydrate by 0.00364, the total acidity with reference to HCl is obtained.

The following table serves for conversion of normal sodium hydrate into HCl.

Table for the Conversion of Normal Sodium Hydrate (Cubic Centimeters) into HCl (per Mille).

0.1	0.0365	2.1	0.7665	4.1	1.4965	6.1	2 2265	8.1	2.9565
0.2	0.0730	2.2	0.8030	4.2	1.5330	6.2	2.2630	8.2	2.9930
0.3	0.1095	2.3	0.8395	4.3	1.5695	6.3	2.2995	8.3	3.0295
0.4	0.1460	2.4	0.8760	4.4	1.6060	6.4	2.3360	8.4	3.0660
0.5	0.1825	2.5	0.9125	4.5	1.6425	6.5	2.3725	8.5	3.1025
0.6	0.2190	2.6	0.9490	4.6	1.6790	6.6	2.4090	8.6	3.1390
0.7	0.2555	2.7	0.9855	4.7	1.7155	6.7	2.4455	8.7	3.1855
0.8	0.2920	2.8	1.0220	4.8	1.7520	6.8	2.4820	8.8	3.2120
0.9	0.3285	2.9	1.0585	4.9	1.7885	6 9	2.5185	8.9	3 2485
1.0	0.3650	3.0	1.0950	5.0	1.8250	7.0	2.5550	9.0	3.2850
1.1	0.4015	3.1	1.1315	5.1	1.8615	7 1	2.5915	9.1	3.3215
1.2	0.4380	3.2	1.1680	5.2	1.8980	7.2	2.6280	9.2	3.3580
1.3	0.4745	3.3	1.2045	5.3	1.9345	7.3	2.6645	9.3	3.3945
1.4	0.5110	3.4	1.2410	5.4	1.9710	7.4	2.7011	9.4	3.4310
1 5	0.5475	3.5	1.2775	5.5	2.0075	7.5	2 7375	9 5	3.4675
1.6	0.5840	3.6	1.3140	5.6	2.0440	7.6	2.7740	9.6	3 5040
1.7	0.6205	3.7	1.3505	5.7	2.0805	7.7	2.8105	9.7	3.5405
1.8	0.6570	3.8	1.3870	5.8	2.1170	7.8	2.8470	9.8	3.5770
1.9	0.6935	3.9	1.4235	5.9	2.1535	7.9	2.8835	9.9	3.6135
2.0	0.7300	4.0	1.4600	6.0	2.1900	8 0	2.9200	10.0	3.6500

The methods described will usually meet the demands of practice. That they are not absolutely exact is clear from what

has been stated. If, however, the presence of organic acids is considered and this eliminated from the estimation, the methods will give information in reference to the glandular function of the stomach.

Higher amounts can be determined by the following methods:—

5. Töpfer's Method.

Töpfer estimates the free HCl with 0.5-per-cent. solution of dimethyl-amido-azo-benzol. Even in the presence of minute amounts of HCl the yellow color of the reagent assumes a reddish tinge, while the organic acids alter the color only when present in excess of 0.5 per cent., and proteid bodies only in still higher concentration.

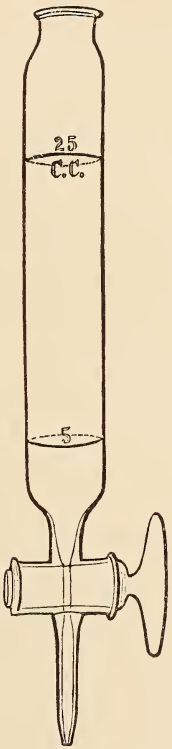
For the estimation of the loosely combined HCl Töpfer employs sodium alizarin sulphonate. Titration by addition of 3 or 4 drops of 1-per-cent. watery solution of alizarin is continued until the first appearance of a distinct violet color. If the value thus obtained is subtracted from the value of the total acidity obtained by titration with phenolphthalein (page 256), the amount of the loosely combined HCl is found.

Determination of Lactic Acid According to Method of Uffelmann.

In testing for lactic acid it is necessary to determine that which has been introduced with the food and that formed in the stomach. Boas has called attention to the fact that all kinds of bakery products contain lactic acid. This acid is also ingested as sarcolactic acid, and with such foodstuffs as sour milk, sauerkraut, and sour gherkins as fermentative lactic acid. It is, therefore, advisable for *exact* analysis to employ a test-meal free from lactic acid. A soup prepared with Knorr's oatmeal, seasoned with salt, is well adapted for this purpose.

Demonstration.—1. A dilute, nearly colorless solution of neutral iron chlorid is turned canary- or lemon- yellow in the presence of lactic acid in amounts exceeding 0.3 per mille. 2. An amethyst-blue solution of iron chlorid and carbolic acid (best prepared by addition of a few drops of dilute liquor ferri sesquichlorati to a 2- to 5-per-cent. solution of carbolic acid) is turned *yellow* by lactic acid.

The reactions are not absolutely decisive, because, in addition to lactic acid and its salts, phosphoric acid salts, butyric acid, sugar, and alcohol produce a similar change of color in the reagent. The test is free from objection only when it is made with a watery solution of the residue obtained by extracting the filtrate with ether and then evaporating the ether. The first test



[Fig. 99.—Strauss's Separating Funnel for Lactic Acid Test.]

can be made by adding the reagent directly to the ether without evaporation. After thorough shaking a *yellowish* precipitate is formed if lactic acid is present.

In hyperacidity due to HCl the lactic acid reaction may be obscured. According to Haas, it is advisable in such cases gradually to dilute the filtrate with water and then make the above test.

In cases of accidental admixture of large amounts of saliva, a *brownish color* may be obtained with Uffelmann's test, owing to the presence of sulphocyanate salts.

The following method for the *quantitative* estimation of lactic acid has been recommended by Strauss: A separating funnel (Fig. 99), with marks at 5 cubic centimeters and 25 cubic centimeters, is filled to the first mark with gastric juice and then to the second with ether. After thoroughly shaking, the fluid is allowed to flow out to the first mark (5 cubic centimeters), then filled with water to the second mark (25 cubic centimeters). Two drops of a 10-per-cent. solution of iron chlorid are then added. A beautiful green color appears in the presence of amounts exceeding 0.5 per mille. In amounts below 0.25 per mille a change of color is scarcely perceptible.

High percentages of lactic acid occur especially in *carcinoma of the stomach,* irrespective of the food ingested. Food plays an important rôle in so far as pronounced lactic acid fermentation may be induced as the result of the introduction of active lactic acid bacilli, especially when ingested with sour milk, buttermilk, sauerkraut, etc. The bacillus acidi lactici of Hüppe is a non-motile rod, about 1 to 1.5 μ long and 0.3 to 0.4 μ thick. It forms lactic acid from cane- and milk- sugar, with the liberation of CO_2.

Large series of statistics have shown that:—

84.4 per cent. of all gastric diseases accompanied by lactic acid fermentation are referable to carcinoma, and

73.5 per cent. of all gastric carcinomata show lactic acid fermentation.

Pathologic lactic acid fermentation is apparently favored by the absence of free HCl, motor insufficiency, and the not infrequently coexistent diminution of ferment secretion.

Absence of lactic acid fermentation does not contra-indicate carcinoma.

Fatty acids, especially butyric acid, color Uffelmann's reagent a fawn-yellow in amounts of 0.5 per mille.

Free *fatty* and *acetic* acids are best detected by their odor. According to Leo, their presence can also be demonstrated with sufficient accuracy for practical purposes by heating 10 cubic

centimeters of the gastric contents in a test-tube and watching for reddening of blue litmus-paper held at the mouth of the tube.

The presence of *acetic acid* can be demonstrated as follows: Some of the unfiltered gastric contents is shaken with acid-free ether and the latter evaporated. The residue is then dissolved in a few drops of water and neutralized with dilute soda solution. If this is now heated with sulphuric acid and alcohol, the pungent odor of acetic acid will be produced if this acid is present.

For the detection of *butyric acid* evaporate in the same manner with ether, and then add *calcium chlorid* to the watery solution of the residue. The butyric acid present separates in small oil-droplets, which give off the characteristic odor of butyric acid.

Test for Pepsin.

The secretion *(pepsinogen)* elaborated by the chief, or central, cells is transformed by *free* HCl into pepsin, which is essential for the conversion of albumin and colloid into a soluble state. In the presence of free HCl any further examination is superfluous. If free acid is absent, then the following method will demonstrate the presence of pepsin: 10 cubic centimeters of filtered chyme are placed in a test-tube and acidulated with 2 drops of officinal hydrochloric acid. A fragment of fibrin or albumin is added and the test-tube placed in an incubator at a temperature of 37.5° C. The early solution of the albumin confirms the presence of pepsin.

The following method of Hammerschlag appears to be well adapted for this purpose: Take two test-tubes, each containing 10 cubic centimeters of a 1-per-cent. solution of albumin, to which 4 per mille of HCl is added. To one of them add 5 cubic centimeters of the filtered gastric contents and to the other 5 cubic centimeters of distilled water. Now place both tubes in an incubator for one hour. At the expiration of this time the amount of albumin in both tubes is determined with Esbach's albuminometers. The difference between the amounts thus obtained represents the quantity of digested albumin.

The peptic power is expressed by the percentage ratio of digested albumin to the amount of albumin originally present in the mixture. In healthy subjects 80 to 90 per cent. is usually obtained.

Test for Rennet.

The demonstration of lab- [rennet] or milk-curdling ferment, which is secreted by the lab-glands and *causes coagulation of milk,* is of less importance. Place in an incubator from 5 to 10 cubic centimeters of milk, to which 3 to 5 drops of the filtrate have been added. If the milk coagulates after from ten to fifteen minutes, lab- [rennet] ferment is certainly present (Leo).

A positive reaction indicates normal lab-gland activity, while a negative result points to serious degeneration of the glandular system, especially if the secretion of pepsin is also disturbed.

Tests to Determine Proteid Digestion.

The following tests may be employed to determine the proteid digestion:—

The abundant presence of **syntonin** is shown by a cloudiness on careful neutralization of the filtrate. The "neutralization precipitate" is redissolved by acid in excess.

Propepton (hemialbumose) gives a cloudiness with concentrated acetic acid and sodium chlorid solution. The cloudiness disappears on heating and reappears on cooling.

If the precipitable albumin is removed by boiling (propepton and pepton do not coagulate on heating), a purple-red will appear in the presence of propepton and pepton in alkaline solution on adddition of copper sulphate (biuret reaction). Albumin and syntonin are turned violet-blue by this test.

In order to determine positively the presence of *pepton* previous precipitation of the *propepton* (see page 298) is necessary. The biuret reaction occurring after such removal is decisive.

Testing of Starch Digestion.

Starch is converted into dextrose (grape-sugar) by the action of the salivary ferment (ptyalin). As intermediate products the dextrins—viz.: erythrodextrin and achroödextrin and especially *maltose*—are of importance. Only the smallest percentage of the starch is converted into dextrose.

The most convenient *reagent* is potassium iodid (Lugol's solution). If on addition of this to the filtrate a blue (starch)

or purple (or violet) color (erythrodextrin) occurs, then *starch conversion is insufficient.*

Achroödextrin, maltose, and dextrose are not altered by iodin. Nylander's test is indicated for the detection of slight amounts of sugar.

Cane-sugar ingested with the food is converted in the stomach into dextrose and levulose, with or without the aid of free HCl. It is usually quickly absorbed.

II. Testing the Motility of the Stomach.

1. Method of Leube.—Six hours after a test-meal [page 252] the stomach is washed out with 1 liter of water. If appreciable amounts of food are absent from the washings, the motor power is normal.

2. Salol Test of Ewald-Sievers.—This test is based upon the theory that salol can be converted only in the alkaline juice of the small intestine into its components phenol and salicylic acid, and that the demonstration of the latter in the urine indicates the passage of the salol from the stomach. The urine voided from time to time is tested for the iron chlorid reaction (see page 327). The salol is usually administered shortly after meals in 1-gram capsules. As a rule, the reaction is distinctly shown as early as from three-fourths to one or one and one-fourth hours after administration.

3. Oil Test of Klemperer. — An accurately determined amount of olive-oil (105 grams), which is not absorbed and only exceptionally altered by the stomach, is administered upon an empty stomach through an esophageal tube. The amount remaining in the stomach after two hours is removed by a stomach-tube and lavage. The oil and water are separated by a separating funnel. The oil is then taken up with ether, and after evaporation of the ether the oil thus obtained is weighed. Klemperer found a 20- to 30-gram residue in healthy subjects.

Critical Analysis of the Above Methods.—It should be emphasized that none of the procedures given can be designated as exact. In practice, Leube's method is preferable, because of its greater accuracy. Usually, however, the stomach washings obtained after a test-breakfast offer sufficient information. If the test-breakfast has passed the pylorus after one and one-half

hours, the gastric motility is usually normal. An exception is offered only by cases of tympany of the stomach, which are characterized by other signs. In these cases, under certain circumstances, nothing can be determined if the lavage gives remains of food. The oil method of Klemperer is too complicated. The salol test is uncertain, because the salol is occasionally also converted by the gastric mucus, and, on the other hand, the reaction may be delayed by intestinal disturbances.

Large amounts of residue always indicate a *diminution* of the motor power. As a rule, intense stagnation and decomposition occur. Sometimes there is only moderate stagnation, but *disagreeable to offensive odor.* According to the author's experience, this is of great value in the *early diagnosis of carcinoma of the stomach.*

III. Testing Absorption.

Testing the absorption, according to Penzoldt and Faber: 0.2 gram of chemically pure potassium iodid is administered in gelatin capsules shortly before mealtime. At intervals of two to three minutes the saliva is tested for iodin with starch paper and fuming nitric acid. In healthy subjects a violet reaction is shown after from six and one-half to eleven minutes, and after from seven and one-half to fifteen minutes a bluish reaction. When given after a meal, the occurrence of the reaction is decidedly delayed. A great delay in the reaction is observed in all gastric diseases. In ectasis Zweifel found the reaction only after two hours. The delay was variable in gastric ulcer.

Boas and many others dispute the reliability of the method, since normal periods of reaction were also observed in ectasis and chronic gastritis.

4. FINDINGS IN AFFECTIONS OF THE INTESTINES.

Intestinal discharges may be inspected macroscopically or examined with the microscope. Inspection frequently confirms a diagnosis based upon other clinical signs, and sometimes it is the *only means* by which a decision can be reached.

Macroscopic examination shows the following:—

The feces of a *healthy individual* are of a light- or dark-brown color, cylindric in form, firm in consistence, and usually

alkaline in reaction. The color is light yellow in children because of the large amount of milk composing their diet. In healthy adults, also, the stools may become dark brown or black through the agency of foods (red wine, huckleberries) and medicines (iron, bismuth subnitrate, through the sulphur compounds formed by them). The dejecta are colored yellow after ingestion of rhubarb, santonin, and senna, and green after calomel. The normal fecal casts usually show fissures and indentations, which indicate their formation from individual scybala. The alvine discharge often appears in the form of masses resembling sheep-manure, without the existence of any pathologic alteration of the intestine.

In **diseases** of the intestine the **quantity, form,** and **color** of the feces may be decidedly altered. Instead of a single stool usually amounting to from 100 to 200 grams, the dejections may be very frequent—10 to 20—and amount to as much as 1000 grams. The cylindric forms disappear; the stool becomes mushy, paplike, or watery. Undigested remnants of food (fragments of potato, vegetables, etc.) can be recognized with the naked eye. These stools may be light or dark in color.

In **biliary congestion** the stools are grayish yellow or clay-like. They are deep brown or black (so-called carbonized stool) in obstinate **constipation.** Fresh blood may be passed with the dejecta in **hemorrhage** into the lower portion of the intestine. When the point of bleeding is located higher up, the stools are usually strikingly altered—dark-brown to tar colored. The latter color is present in stools following gastric hemorrhage. In **cholera** rice-water or souplike evacuations occur. In many forms of **enteric catarrh** (especially in children) the stools have a bile-green color.

Mucous shreds or flocculi are observed in the stools of healthy individuals only when the feces are very hard and firm. Large *mucous shreds* are often mixed with *thin* dejecta, or large *gelatinous* masses of mucus may be expelled with or without feces (colitis, cholera, dysentery, etc.). Tenacious, glassy mucus occasionally adheres to a single firm stool (catarrh of lower colon and rectum), or pure mucus (proctitis), or long, ribbonlike or tubular-formed mucous coagula are discharged with the stools (see "Enteritis Membranacea").

Sagolike bodies, the vegetable origin of which can be determined by the microscope, may be mistaken for masses of mucus.

The usually alkaline *reaction* of the feces, which often varies even in healthy individuals, may become acid, especially in children suffering from acute catarrhal enteritis. The reaction is of no diagnostic significance. The well-known "fecal odor" becomes in many diseases stinkingly putrid (cancer, etc.) or disappears entirely (dysentery).

In addition to many foreign bodies small and large **gall-stones** and **worms** (see page 116) may appear in the dejecta and be of valuable diagnostic significance.

Bile-concretions occur in the feces as true stones the size of a pigeon's egg and larger, or in the form of gravel. It is necessary to sift and wash the feces in order to detect the smaller stones. The stones have a polygonal or a tablet form. They are usually soft and of a yellowish, gray-white, or brown color. They are sometimes homogeneous, and on fracture present a distinctly crystalline surface. They may also be of composite formation, presenting a dark nucleus, radiate lamellæ, or often of a smooth, white or greenish color with a roughened, grayish-black cortex. **Cholesterin** and **bilirubin-calcium** are the chief constituents of stones. The rare **pure cholesterin calculi** are pure white or yellowish white, usually smooth, translucent, and sometimes show a glistening, pearly surface, owing to superficial deposits of cholesterin crystals. The much more common **cholesterin-bilirubin** stones are sometimes yellow or dark brown, sometimes greenish brown, and also usually have a smooth surface. Calcium carbonate calculi, on the other hand, are often roughened.

Gall-stones occur more frequently in women than in men (four to five times), and particularly in women who have borne children. They are rare prior to the thirtieth year, more frequent after thirty, and very frequent in people over sixty years of age. A desquamative angiocholitis is the primary disturbance.

Microscopy of the intestinal discharges is very repulsive, and in many cases can be practiced only with certain precautions. Preventive measures are indicated at times to avoid danger from infection, and many aids are necessary on account of the intolerable odor. In the examination of thin stools it is advisable to cover the specimen of feces in a conic glass with a layer of ether. The odor is thus greatly diminished. For examination some of the sediment in the bottom of a conic glass or a definite portion distinguishable to the naked eye is selected with a pipette; or

the stool may be spread upon a plate and examined for definite particles.

Under **normal** conditions there will be found (Fig. 100) :—

1. Food-remnants.—*Muscle-fibers,* recognizable by distinct transverse striations, are not often found; *starch-remnants,* very seldom; more frequently, plant-cells of salad, spinach, and fruits; *milk-remnants* in the form of yellow-white flocculi; finally, *fat,* more often in crystalline than in globular form.

2. Crystals and Salts.—Triple phosphate, in coffin-lid form, and large and small rosettes of neutral calcium phosphate are of most frequent occurrence. Calcium oxalate in envelope form is rarer (Fig.

Fig. 100.—Stool. Collective Microscopic Picture. × 350.
(Partly after *Nothnagel.*)

m, Muscle-fiber. *e,* Intestinal epithelium. *ve,* The same, "broken down." *c,* Clostridium butyricum. *h,* Yeast. *p,* Vegetable cells. *t,* Triple phosphate.

128). Lime salts which are stained yellow with bile-coloring matter and give the well-known reaction on addition of nitric acid are frequent. Cholesterin crystals are scarce.

3. Epithelial Cells are usually absent; but a few cells may be mechanically dislodged only from the squamous-celled covering of the lower rectum by the passage of firm feces.

4. Bacteria are always found in large numbers. In addition to elliptic *yeast-cells,* which are usually of a yellow tint, and the long, motile rods and large masses of the *bacillus subtilis,* many forms of cocci and bacilli, which stain blue when treated with Lugol's solution, are deserving of attention. Among them the **clostridium butyricum,** thoroughly investigated by Nothnagel, appears in the form of broad

rods with rounded ends or as elliptic or spindle-shaped bodies. Their size and arrangement varies. They may occur singly or in the form of zoöglœæ. They stain blue or violet *in toto* or only in their central portions with Lugol's solution. They are more numerous in vegetable diet than in one of proteid. As Breiger has shown, they give rise to butyric acid fermentation.

In **pathologic states** of the intestine **microscopy** shows (Fig. 100) :—

Aside from the admixtures of undigested food, which are macroscopically recognizable in severe disturbances, there is noted in milder cases a considerable increase of muscle-fibers and the *appearance of undissolved starch, which normally is rarely present. Its abundant occurrence indicates the existence of serious catarrh.* Furthermore, casein, fat, and triple phosphates are present in large quantity. Cholesterin and hematoïdin crystals are usually rare. Delicate octahedra, morphologically and chemically resembling Charcot-Leyden crystals, are decidedly more frequent. These crystals are occasionally found in stools of patients suffering from typhoid fever, dysentery, and phthisis, and they appear almost constantly in anchylostomiasis, always in anguilluliasis, frequently in ascaris lumbricoides, oxyuris, and tæniæ saginata and solium. They are sparingly found in trichocephalus, and they were totally absent in the cases of tænia nana so rarely observed in Germany (Leichtenstern). According to Leichtenstern, in every case in which Charcot's crystals are found in the feces the presence of worms should be assumed as very probable. On the other hand, the absence of the crystals does not preclude helminthiasis.

The facts that the crystals are most numerous in that portion of the intestine in which anchylostomum is usually located (upper *ileum*, not duodenum); that they are very abundant in the slimy, bile-stained stools induced by drastics in anguilluliasis; that their appearance, even though seldom, in the stools some time after an anthelmintic course has been pursued always points to incomplete expulsion of worms (retention of the particularly tenacious male anchylostomum or of tapeworm head), all indicate that the crystals are formed at the seat of the parasites (Leichtenstern).

For the detection of intestinal parasites it is necessary not only to examine for discharged worms, worm-segments, and embryos, but especially for the ova shown in Fig. 56.

The great importance of examinations for the detection of worms is shown by the fact that by this means it has repeatedly been possible not only to demonstrate for the first time the presence of parasites, but by their expulsion to remove severe pathologic conditions (see page 121). The author observed a lady, a resident of St. Petersburg, who, as a child, had often eaten insufficiently smoked pike in Finland, and for years had suffered from severe anemia. She was in a condition of serious anemia and debility; numerous ova of bothriocephalus were found in the stools. Expulsion of a worm eight meters in length was followed by steady improvement, and final complete cure was established by the administration of iron. The frequency of parasites is shown by the statistics of Heisig, who was able to demonstrate parasitic ova in the stools of 119 persons out of 230 examined (51.7 per cent.).

In many instances the presence of worms is indicated by no demonstrable macroscopic alterations of the stools. That chronic diarrhea, which may cease after expulsion of tapeworms, is occasionally present has already been mentioned (see "Tænia Nana," page 120). Various *infusoria* have recently occasionally been found in chronic diarrhea in such large numbers as to be of significance. While, on the one hand, proof of the etiologic relation of the infusoria to the *origin* of the disease could not be established, on the other, there was no doubt that the infusoria were responsible for the *perpetuation of the diarrhea.* In addition to the megastoma entericum mentioned on page 107, cercomonas, trichomonas, and peculiar pear-shaped infusoria have been found in such conditions. In this connection the author would again draw attention to the significance in dysentery of the amœba referred to on page 104.

Quincke and Roos, who first directed attention to this subject, also found animal parasites in two cases of *dysentery.* In the first case, imported from Naples, a form identical with the *amœba Lösch* (page 104) was found. It produced fatal dysentery in cats. In the second case, originating in Kiel, a much less infectious amœba was observed. The author has observed amœba in the *freshly discharged,* bloody-purulent flocculi in several cases of tropical dysentery.

Of the *pathogenic bacteria* occurring in the intestinal discharges, the bacilli of tuberculosis, typhoid fever, and cholera are

deserving of special consideration (see pages 47 *et seq.*). In certain cases of acute enteritis streptococci occur in large numbers in the dejecta. For this and other reasons they are to be regarded as an etiologic factor in this disease (Escherich). It may here be remarked that Kruse, Shiga, and others [Duvall and Bassett (page 276)] have found a specific bacillus as the exciter of dysentery. It should not be forgotten that, under certain circumstances, the *gonococcus* also may be present. It may also be stated that the diarrheal stools of infants, especially the mucous admixtures, very frequently contain *spirilla* the source of which is not certain. At necropsies made by Escherich upon such children these organisms were found almost exclusively in the mucous deposits in the colon and especially the cecum.

Discharge of admixed *mucus* is of great clinical significance. Mucus visible to the naked eye can readily and positively be identified by its chemic behavior. It also occurs in the form of *yellowish-brown to dark-green granules,* as first pointed out by Nothnagel. If these granules are crushed beneath a cover-glass, they spread out into a uniform yellow mass, while the yellow bodies, resembling sago or frog-spawn, which usually consist of vegetable remnants and water, always remain in fragments. They are neither dissolved nor stained by water, ether, iodin, or osmic acid. On addition of nitric acid they give a distinct reaction for bile-coloring matter. They present no special structure, and always indicate catarrh of the ileum and upper portion of the colon. They also occur in pure ileitis. The active reaction for bile-coloring matter with regard to the presence of mucus is of itself evidence of the existence of catarrh of the ileum, for the reason that bile-pigment is normally found *only* in the ileum, *never* in the colon, and can, therefore, occur in the feces only when there is very active peristalsis of the ileum and colon. If with the coloring matter mucus also occurs, proof of catarrhal ileitis is established.

Cylindric epithelial cells imbedded in mucus are of frequent occurrence in different pathologic conditions of the intestine. The cell form is usually altered, swollen, or shrunken. The protoplasm is granular from fatty degeneration, and the contour and nucleus preserved. Unaltered epithelial cells are found exclusively in large mucous shreds. Under the term "broken-down"

epithelia Nothnagel has described spindle-formed, slightly glistening bodies which have been altered by desiccation. They occur *more frequently in dry than in diarrheal stools.*

In addition to epithelial cells *leucocytes* of variable size are usually present. As has already been stated, admixture of *pus* is found in the feces only in ulcerative processes of the intestinal canal or adjacent parts.

5. CHARACTER OF THE DEJECTA IN CERTAIN AFFECTIONS.

1. In **Acute Intestinal Catarrh** the number of stools is more or less increased and the consistency is pasty or liquid. According to the seat of the catarrh, certain differences are manifest:—

(a) If *the ileum only* is affected, frequent, thin evacuations, mixed with macroscopic bile-stained mucus inclosing numerous cylindric epithelial cells, occur; the yellow mucous granules mentioned above (Nothnagel's) are also often observed.

(b) In catarrh of the *upper portion of the colon,* which is usually associated with catarrh of the small intestine, the thoroughly mixed, liquid, souplike dejecta contain mucus in *microscopic* form *only.*

(c) In *catarrh of the rectum (proctitis) pure gelatinoid mucus* is often expelled.

(d) In catarrh of the *whole large intestine* the liquid, souplike stools contain *macroscopic* masses of mucus which are not bile-stained.

2. **Chronic Intestinal Catarrh** generally presents the following features:—

(a) Chronic *catarrh of the ileum* does not occur *alone.* Combined with *catarrh of the colon* it induces frequent daily liquid stools, containing bile-stained mucus, *yellow* mucous granules, etc.

(b) When limited to the *colon* there is almost always a disposition to constipation of several days' duration, which may be interrupted at regular or quite irregular intervals by diarrhea.

(c) In implication of the *rectum,* with or without disturbances of the lower colon, the feces are imbedded in mucus.

3. **Nervous Diarrhea** is of frequent occurrence in neurasthenics, and may be attended by from 6 to 8 or 10 alternately

solid and liquid stools daily. Now and then at mealtimes there is felt a sudden desire to defecate. The abundant *bilious* admixtures, which are often frequent, indicate abnormal peristalsis in the small and large intestine.

4. **Enteritis Membranacea.**—In this affection there are discharged at intervals, with or without stools and not infrequently accompanied by violent colicky pains (hence "mucous colic"), membranous, ribbonlike or tubular formations (membranous or tubular enteritis). Their color is dirty white and their length often considerable (in a large series of cases the author found them to measure from six to twenty centimeters). The discharges may be repeated daily for weeks or only a few times in a year. They are extremely rare in children or neurasthenic men, but much more frequent in *nervous* or *hysteric women.* Not infrequently a tendency to constipation coexists.

Microscopically, a delicate, striated basement substance, which may here and there present glistening, fibrinlike fibrillations, is observed in all cases. This is usually clouded throughout by acetic acid—an indication that it consists of mucus. Very numerous, greatly altered cylindric epithelial cells and leucocytes are also often present. Triple phosphate and cholesterin crystals are occasionally found. The *chemic* behavior of this basement substance shows that it is composed chiefly of *mucus,* in addition to which an albuminoid body may occur. The coagula are almost entirely dissolved by caustic potash. Addition of acetic acid to the filtrate produces intense clouding, which almost wholly disappears on adding an excess of acetic acid.

It can scarcely be doubted that this affection, which probably attacks nervous subjects exclusively, is a genuine secretion neurosis in which the normal mucous secretion is augmented. If in such individuals a certain sluggishness of the stools with spasmodic peristalsis of the colon is also present, as, indeed, is often the case, the mucus may, as Marchand first pointed out, be molded between the longitudinal folds of the mucosa of the colon into strings, membranes, or even tubular-formed masses. As a matter of curiosity, I may here mention that one of my patients, suffering from this malady, repeatedly submitted herself to tapeworm cures upon the advice of a quack.

5. **Intestinal Ulcers** are very often accompanied by *diarrhea,* but the latter may occasionally be absent even in extensive ulceration. *Blood* or *pus* mixed with chronic diarrheal dejecta is

strongly suggestive of ulceration. It should be especially noted that *ulcers of the ileum,* the sanguino-purulent secretion of which may not appear in the stools, are generally *unattended* by diarrhea. On the other hand, ulceration in the *lower portion of the colon and rectum* is always accompanied by diarrhea. On careful examination of such dejecta, *blood and pus are very rarely absent if dysenteric ulceration is present. They may not be present in tubercular and catarrhal (follicular) ulcerations.* "Small, grayish-white clumps," consisting of closely packed pus-corpuscles, are only of occasional occurrence. The *larger* masses resembling swollen sago granules, previously mentioned as indicative of follicular ulcer, consist, as Nothnagel first noted, almost always of particles of starch or fruit.

Besides blood and pus, the "tissue-shreds" found almost exclusively in dysentery, mingled with the diarrheal stools, are of important diagnostic significance.

6. **Atrophy of the Intestinal Mucosa,** when it affects limited areas of the bowel, may be wholly unattended by symptoms. Diarrhea occurs in atrophy of the mucosa of the colon. However, the stools contain neither macroscopic nor microscopic evidence of mucus.

7. In **icterus catarrhalis** the stools are usually clay-colored, firm, and richly fatty. The fat is usually present in the form of tufts or sheaves of needlelike crystals, which, according to Osterlein's researches, probably represent lime and magnesium salts of higher fatty acids. The crystals remain unaltered even after twelve hours' treatment with sulphuric, nitric, hydrochloric, and acetic acids. They also resist the action of ammonia, potassium, and sodium. They thus differ very decidedly from Charcot-Leyden crystals, which immediately disappear on treatment with these reagents.

8. In fatty and amyloid **degeneration of the liver** and **hepatic cirrhosis** quite similar oligocholic or acholic stools also occur unattended by icterus or bile-stained urine.

9. In pronounced **intestinal tuberculosis** *tubercle bacilli* are nearly always found in the *stools.* Their presence is directly referable to intestinal tuberculosis. It should not be forgotten, however, that sputum containing bacilli is swallowed by pulmonary consumptives, and the bacilli may appear in the stools without the existence of intestinal tuberculosis. This question is still in dispute. In individual cases the author unqualifiedly agrees with Lichtheim, and would diagnosticate intestinal tuberculosis on detection of the bacilli in the feces.

According to Lichtheim, it is better to omit contrast staining, for the reason that the innumerable nonpathogenic bacteria present in the stools (see page 269) are stained and render the tubercle bacilli, which

are usually few in number, more difficult to find than when the simple "specific" method of staining is employed (see page 49).

Therefore, dry preparations made from the mucus or, better still when present, muco-purulent admixtures should be used. This is stained only in carbol fuchsin or gentian-violet-anilin-water solution and decolored with hydrochloric or nitric acid and 70-per-cent. alcohol (see pages 49 *et seq.*). Decoloration must be so thoroughly done that all possibility of confusion with *smegma* (pseudotubercle) bacilli is excluded.

10. **Dysentery.**—The stools are extremely frequent (10, 20, and more in twenty-four hours) and usually accompanied by severe pain and tenesmus. While only a small quantity is discharged with each dejection, taken collectively the amount is often considerable (1000 to 1800 cubic centimeters, according to the author's observations). The dejecta preserve their fecal consistence and odor only in the earliest stage. When the disease is fully established, they are composed only of *mucus*, *blood*, *pus*, and tissue-shreds. According to the proportion of these constituents we distinguish (just as with the sputum) simple mucous, muco-hemorrhagic, pure hemorrhagic, and pure purulent stools. Muco-purulent-hemorrhagic and other mixed forms also are not infrequently observed. In the beginning mucus predominates. It appears as a thin, tremulous, yellow-stained colloid which either incloses particles of feces still present in the early stages or is mixed with them in large masses. From the very beginning the mucus is spotted and streaked with blood. "Mucous shreds" in the form of flat coagula, which cover the stools, are not rarely observed.

The hemorrhagic elements in the beginning may be simply an indication of hyperemia of the mucous membrane of the colon; in the later stages these admixtures, especially those of a purely hemorrhagic type, are derived, like the pus, from the existing ulcerations. In more extensive and deep destruction of the intestinal mucosa, unquestionable tissue-fragments appear in the *putrid*, stinking, dirty-browish-red or blackish stools.

The **microscope** readily reveals mucus and pus by morphologic and microchemic (acetic acid reaction of the mucus) characteristics. Fresh blood is recognized by the presence of red blood-cells. Old blood is often demonstrable only by means of chemic and spectroscopic procedures (see page 189). The bloody-infiltrated mucous clumps often contain the *amœbæ* described as the cause of dysentery (see page 104, Fig. 53). [A bacillus discovered by Shiga, of Tokio, and confirmed by Flexner and others, has recently been described as an etiologic factor in tropical forms of dysentery. It is also claimed that this micro-organism is the chief etiologic agent in the summer dysentery of children.—Brooks.]

11. The firm and formed stools present in the early stage of **typhoid fever**, usually toward the end of the first week of the disease, become thin and watery and still have a distinct brownish color. The diarrhea, which then begins and continues during almost the whole period of the fever, is manifested by five, six, and more light-brown, pale-yellow, and

yellow-tinged stools, which separate into two layers on standing. The lower one contains flocculent and lumpy yellow masses from which the upper, more or less cloudy, brownish-yellow-colored, watery stratum has separated. This "*pea-soup-like*" *stool* loses its light-grayish-yellow color only toward the end of the disease, during the gradual decline of the fever. It then becomes brownish and gradually more firm until of normal consistence.

In the sediment of the pea-soup-colored stool there are found, in addition to putrefactive bacteria, a varying number of round cells, depending upon the amount of mucus, and many crystals (triple phosphate), abundant *bile-pigment*, casein flocculi, and the specific pathogenic *typhoid bacilli* (page 60). *These are recognizable only by culture methods.*

In intestinal hemorrhages, which, as is known, occur from the end of the second to the fourth week in from 6 to 7 per cent. of the cases, pure blood or thick or slightly coagulated dark blood may be sometimes discharged in large quantity. If the hemorrhage is slight or a large amount of blood has been retained for some time in the intestine, the feces may be brownish or even tar colored.

Slight admixtures of blood with the stools not infrequently give warning of an impending severe hemorrhage. Consequently these streaks of blood or blood-stained mucous shreds visible to the naked eye should be attentively watched for and given most careful consideration.

In the stools discharged with severe hemorrhage, the red blood-cells are often still recognizable. In the blood which has been much altered in color, even the "ghosts" of the red blood-corpuscles are absent. Under such circumstances blood-coloring matter must be demonstrated by Teichmann's *hemin test* or by means of the *spectroscope*. By the latter method it must be remembered that the oxyhemoglobin may have been transformed into methemoglobin (page 191).

12. **Cholera.**—The characteristic "rice-water" or "oatmeal-soup-like" stools are usually very frequent and profuse. Owing to the absence of bile-pigment, they are liquid, grayish white, mixed with light-colored flocculi resembling cooked rice, and devoid of fecal odor.

Microscopic examination of a simple, unstained "crush" preparation, made from one of the light-colored mucous flocculi, shows that they are composed of closely arranged, swollen cylindric epithelial cells and mucus, among which are numerous *bacteria of all varieties.*

Consequently the specific infectious agent can rarely be recognized in a dried and stained specimen. *Cultivation* is essential. Koch and numerous other investigators observed on former occasions and also in the severe epidemic at Hamburg, in 1892, a number of cases in which

the comma bacilli (page 70) were present in stained preparations in almost pure culture, and noted especially the characteristic grouping of the bacilli in the mucous flocculi. In some of these cases the diagnosis can be made with great probability without cultivation, because the comma bacilli are distinguished from other comma species by their shorter, thicker, and more curved form and their characteristic arrangement in clumps.

13. In **syphilis of the rectum** mucus and blood are not infrequently discharged with the feces.

14. In **cancer of the rectum** frequent, nonfeculent discharge of blood and mucus accompanied by tenesmus is particularly characteristic. When the seat of cancer is higher up, putrid, stinking dejecta, very rarely containing cancer-fragments, may support a diagnosis. On the other hand, tapelike or sheep-manure-like stools are of no differential diagnostic significance.

15. **Intussusceptions** of the intestine lead to bloody-mucoid dejecta; more rarely to expulsion of necrotic portions of intestine. Embolism of the mesenteric artery, severe congestion of the portal vein, and scorbutus also cause bloody stools.

PLATE I.

From Nature by Dr. J. Vogel.

[VOGEL'S SCALE OF URINE TINTS.]

V. EXAMINATION OF THE URINE.

THE PROPERTIES OF NORMAL URINE.

Examination of the urine reveals valuable information, with reference not only to general metabolism, but also to the condition of all those organs concerned in the elaboration and discharge of this important secretion. A high estimate has, therefore, been placed upon careful examination of the urine since olden times. Examination is directed chiefly to the *chemic* and *microscopic* characteristics. Before describing the methods of examination and the conclusions to be drawn from them, the properties of **normal urine** will be briefly considered.

The **total quantity** of urine voided in twenty-four hours by the healthy male averages from 1500 to 2000 cubic centimeters, and by the female, from 1000 to 1500 cubic centimeters. It may be temporarily diminished or increased by bodily exercise, excessive ingestion of fluids, etc., without the existence of pathologic deviations.

Urine is usually perfectly **clear** and transparent, and on shaking shows a whitish foam, which quickly disappears. After long standing there forms at the bottom of the vessel a delicate, whitish cloud *(nebecula),* which is composed of isolated mucous corpuscles (leucocytes), squamous epithelia, and salts. On cooling a reddish sediment composed of sodium urate is not infrequently deposited, which disappears on heating. In faintly acid urine a whitish deposit is formed which is composed of earthy phosphates and does not disappear on heating. Indeed, the phosphates are sometimes precipitated only after heating.

The **color** of the urine may vary from straw-yellow to brick-yellow-red. The more acid the urine, the darker the color.

The almost constant **acid reaction** of healthy urine is due principally to the presence of acid sodium phosphate. Under physiologic conditions—*e.g.,* during preponderance of vegetable diet and abundant consumption of alkaline carbonates, and less often shortly after the principal meal—it may be faintly acid or

even alkaline. The earthy phosphates and earthy carbonates precipitated from such urine spontaneously or only after heating are at once dissolved by *addition of acid*. *Amphoteric* reaction, in which the urine turns blue litmus-paper red and red litmus-paper blue, is rare.

The **specific gravity** usually varies between 1.012 and 1.024. It may be decidedly lowered by ingestion of large amounts of water and increased after profuse sweating. It is *determined* by means of the *urinometer* (Fig. 101)—an araëometer, gauged at 15° C., and graduated from 1.000 to 1.050 or higher.

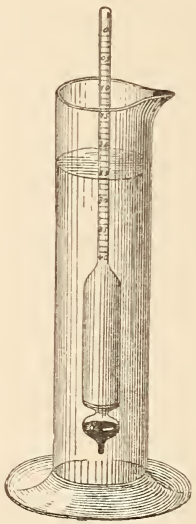


Fig. 101.—Urinometer.

The apparatus is immersed in the urine contained in the cylinder, and the specific gravity read off from the *lower* meniscus. The urine must be absolutely clear, and the urinometer must move freely in the fluid and touch neither the bottom nor sides of the cylinder. The layer of foam which is often present on the top of the fluid and interferes with correct reading should be removed with a glass rod. [For very small amounts of urine, the so-called *specific gravity beads* are very useful in estimating approximately the specific gravity.—Brooks.]

The aromatic **odor of the urine** present under ordinary circumstances may be influenced or increased by certain foods, such as asparagus and the like. A violetlike odor is produced by breathing oil of turpentine (*e.g.*, after polishing of parquet floors).

Among the **organic** constituents excreted daily with the urine urea assumes the first place. The average amount discharged is 30 grams *per diem*. Uric acid is daily excreted in amounts of 0.75 gram. Among the **inorganic** constituents sodium chlorid is present in largest amount, averaging 14 grams. Sulphuric and phosphoric acids are excreted in amounts of 2.5 grams each per day.

CHARACTER OF THE URINE IN DISEASE.

Under pathologic conditions the urine is often decidedly altered. Some changes can be recognized with the naked eye; as a rule, however, careful chemic and microscopic examination is necessary.

The following deviations may be observed with the naked eye: **Increase in quantity** in diabetes insipidus and mellitus, contracted kidney, pyelitis, etc. **Diminution** in febrile diseases, valvular lesions of the heart, many forms of nephritis, in uremia, cholera, etc. **Alterations in appearance and color:** The urine may be cloudy or quite opaque, and instead of a light-yellow hue assume a dark red to inky black or even a milky-white shade. The presence of blood- and bile- coloring matters, indican, melanin, and admixture of pus, as well as a strong precipitate of urates, can sometimes be surmised with greater or less certainty without further examination.

It may be mentioned here that the specific gravity may present great variations between 1.000 and 1.060. Abnormally low specific gravity is observed principally in diabetes insipidus and contracted kidney; very high specific gravity is seen chiefly in diabetes mellitus.

The **acid reaction** is subject to many variations in disease, especially in catarrh of the bladder (see below). It should here be mentioned that after severe vomiting, after lavage of the stomach, and in hyperchlorhydria the urine may become alkaline,

owing either to loss or to too profuse elaboration of hydrochloric acid.

THE MOLECULAR CONCENTRATION OF THE URINE [CRYOSCOPY[1]].

A few remarks upon the molecular concentration of the blood have already been given [page 147]. From the statements there made it is plain that in defective renal function and consequent retention in the blood of substances which should be excreted in the urine, a diminution of the molecules in the urine must occur in the same ratio. In other words, *in disturbed renal function the freezing-point of the urine* (Δ) *rises above the normal.*

It has been shown that in healthy kidneys this varies between 0.87° and 2.43° C., according to the conditions of metabolism. In order to obtain an approximate idea of the actual amount of molecules excreted, the daily amount of urine must, of course, be considered. In order to obtain standard values, the product of Δ and the amount of urine $= \sqrt{}$ (valence number) has been calculated. The figures given by different authorities vary between 766 and 3770. While the practical value of the molecular determination of the urine in internal medicine is limited (because the limits of the normal value, which among other things are decidedly influenced by solid and liquid food, are very variable), the researches of Kummel and Rumpel have shown that cryoscopy is of the greatest advantage in the diagnosis of unilateral renal affections. For this purpose it is necessary to collect the urine of both kidneys separately by ureteral catheterism. The urine of each kidney is then examined in regard to its freezing-point and also its urea and sodium chloride content. If Δ of one kidney shows a normal value, while Δ of the other kidney is under 0.87, this indicates an affection of the latter.

The method of determining the freezing-point of the urine is the same as that for the blood [see page 147].

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[1] From κρύος = ice.

CHEMIC EXAMINATION OF THE URINE.

1. DETECTION OF THE NORMAL CONSTITUENTS OF THE URINE.

Urea.—The urine is evaporated to a thick syrup and extracted with alcohol. It is then filtered, the filtrate evaporated, and the residue dissolved in water and treated with concentrated nitric acid. On cooling, nitrate of urea separates after a time in rhombic or hexagonal plates.

A simple, although not very exact, method of determining the amount of urea has been offered by Esbach, of Paris. So far as the determination of approximate values or obtaining comparative figures in urine secured separately by ureteral catheterization is concerned, the method may be recommended, and is therefore referred to in this place.

[One of the most important procedures in urinary examinations is the **quantitative estimation of urea.** Many methods and apparatus have been devised for this purpose, all of which are more or less practical and convenient. We have found, however, that the Doremus ureometer, especially the recent modification of this instrument, gives results sufficiently accurate for all practical purposes. The construction of the two forms of apparatus is shown in Figs. 102 and 103. The test is based upon the amount of nitrogen-gas liberated from a given quantity of urea. In this test the solution used to decompose the urea is the hypobromite of soda. The method of procedure is as follows:—

Make a 40-per-cent. watery solution of sodium hydrate (pure sticks). The sodium hypobromite test solution is made by adding pure bromin to the sodium hydrate solution in the proportion of 1 part of the former to 10 parts of the latter. The bromin and sodium hydrate solution should be mixed fresh for each test. It is most convenient to pour sufficient sodium hydrate solution into the long arm of the apparatus to reach to the mark = and then add enough water completely to fill the long arm and bend of the tube. The tube should be sufficiently full to avoid entrance of air into the long arm when the instrument is tilted in a direction toward the bulb, as should be the case when making the test. The thumb is now placed over the opening of the tube

and the solution shaken until thoroughly mixed. Now add 1 cubic centimeter of bromin with the cubic centimeter pipette which accompanies the apparatus. The bromin can be made to mix with the dilute sodium hydrate solution by pressing rapidly a number of times upon the rubber bulb of the pipette while the latter is immersed in the solution with the tip directed toward the top of the closed tube. When the bromin is dissolved, again

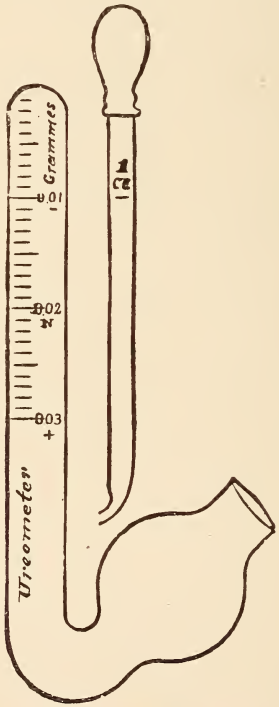


Fig. 102.—Doremus's Ureometer.

place the thumb over the opening of the tube and shake well as before, to insure thorough mixture. Now allow all air to escape from the tube and then slowly and carefully add exactly 1 cubic centimeter of *fresh* urine with the pipette (if old form of apparatus is used), the point of the pipette directed upward toward the top of the closed tube. As soon as the urine comes in contact with the hypobromite solution nitrogen and carbon dioxid

gas are set free and the former collects at the top of the tube. The CO_2 gas is at once absorbed by the excess of sodium hydrate, forming sodium carbonate, and therefore does not interfere with the test. The introduction of the pipette containing the urine requires no small amount of dexterity to avoid loss of gas. Some gas will always be lost, but if the pipette is quickly, but carefully, introduced this loss will not materially interfere with the result. With the modified instrument (Fig. 103), which is to be preferred, this source of error is avoided. In this apparatus the urine is placed in the graduated small, straight tube and the flow controlled by the stopcock. The reading is the same in both apparatus.

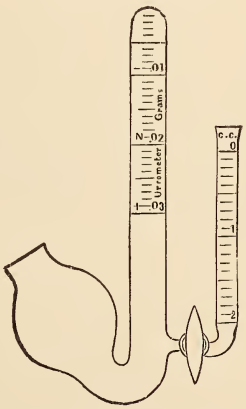


Fig. 103.—Doremus's Ureometer, Improved.

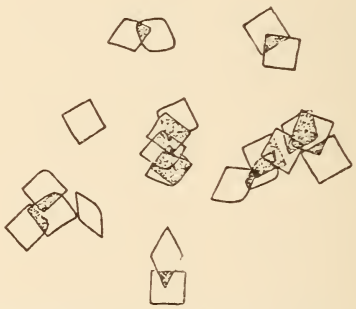
Each mark upon the scale represents 1 milligram of urea per cubic centimeter, or 0.1 per cent. Upon the graduated closed tube to the left of the scale are the marks —, N, and +, which represent minus, normal, and excess, respectively. The instrument is also made with graduations showing the number of grains of urea to the ounce of urine. If the number of cubic centimeters of urine passed in twenty-four hours is multiplied by the percentage obtained with the apparatus, the total amount of urea for twenty-four hours will be secured.

The solution of hypobromite in the tube will indicate 6 per cent. of urea; so that, if a specimen in which not more than 3

per cent. of urea is indicated by the scale was first used, the solution may be used again for a second specimen containing a like amount or less of urea. All that is necessary is to allow the accumulated gas to escape and then proceed as with the first test.—Brooks.]

Urea is diminished in chronic hepatic and renal diseases, principally, however, in acute yellow atrophy of the liver, in which, under certain circumstances, the urea excretion may be wholly suspended and leucin and tyrosin (which see) appear in its place.

Urea is increased by augmented proteid destruction in fever, and also in diabetes mellitus, in which affection most abundant excretion occurs.



[Fig. 103*a*.—Colorless Uric Acid Crystals. × 350. (After *Simon*.)]

Uric Acid.—A portion of the sediment, to which a few drops of nitric acid have been added, is slowly evaporated to dryness upon a porcelain dish. The orange-colored spot thus formed turns a purplish red on addition of ammonia, and blue on subsequent addition of potassium hydrate *(murexid test)*.

Decided increase is observed in gout, leukemia, etc.

[Quantitative Estimation of Uric Acid. — Quite recently Ruhemann presented to the Berlin Medical Society a new method for the *quantitative* estimation of uric acid in the urine, which is based upon the principle of the union of uric acid with iodin and is carried out in the following manner:—

The author has constructed a burette, 25 centimeters in length, with the following divisions: The lowest mark *(S)* shows the height to which the indicator, sulphid of carbon,

should reach. Then follows, to the mark *I*, a space of 2 cubic centimeters' content into which iodin solution is poured. The latter solution is composed of iodin, 1.5; potassium iodid, 1.5; alcohol, 15.0; water, 185.0. Above the mark *I*, at 2.6, begins an empiric scale which, at distances of 0.2 cubic centimeter, gives the uric acid value *pro mille*. It runs from 2.45 to 0.175 grams *pro mille* (see Fig. 104). After the sulphid of carbon and iodin solution have been placed in the burette, observing the precautions laid down by Ruhemann, the urine is slowly added and the mixture vigorously shaken after each addition. The urine is added until the primary brown color gives place to a white one, at which moment the percentage of uric acid is read off by the aid of the figures at the top of the column of fluid. If the urine contains less uric acid than the apparatus will indicate, add the iodin to the mark midway between *I* and *S* and read *half*-values. Alkaline urines should be acidulated with acetic acid, and, if abundant sediment of sodium urate is present, the specimen should first be well shaken. Traces of sugar and albumin do not interfere, but if large amounts of albumin and traces of pus or blood are present, these should be coagulated by heat and then removed by filtration. The procedure requires, on an average, about from thirty to forty-five minutes. Ruhemann claims for his method rapidity and an accuracy equal to that obtained by the weight analysis method.

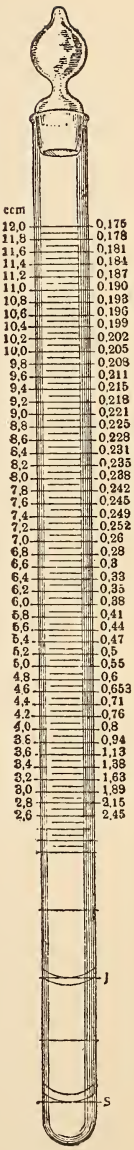


Fig. 104.—Ruhemann's Uricometer.

Clinical Estimation of the Quantity of Purin in the Urine by Means of the Purinometer.—The term "purin bodies" includes all combinations which contain the nucleus C_5N_4. The principal ones are hypoxanthin, uric acid, guanin, and methylxanthin. The purin bodies contained in the urine are most conveniently divided into "exogenous" and "endogenous." The former are

derived from the purin bodies of the foods, and their amount is in direct proportion to the quantity of food taken; the latter are very probably derived from disintegration of the body-nucleins during metabolism.

According to Hall, Berding and others have recently asserted that simple determinations of uric acid are of no practical clinical value, except as an aid in the diagnosis of acute attacks of gout and for the demonstration of the presence of leukemia. When the relation existing between the purin bodies of the foods and of the urine is accurately determined, the advantages of a systematic determination of the *individual* exogenous and endogenous purins under normal and abnormal conditions will be more apparent.

On an average, about 50 per cent. of the purin taken with the food is excreted in the urine during the following twenty-four hours, although in certain individuals the amount varies up to 10 per cent. A knowledge of the amount of endogenous purin and repeated determination of the purin in the urine of patients (together with the use of tables which give the quantitative purin content of ordinary foods) permits exact regulation of the daily activity of metabolism in the body.

The procedure is based upon Camerer's method for the determination of the total amount of purin bodies in the urine. For this purpose two solutions are required:—

SOLUTION I.

Ludwig's magnesia mixture[1]	100 cubic centimeters.
Ammonia (20 per cent.)	100 cubic centimeters.
Talcum	10 grams.

SOLUTION II.

Silver nitrate	1 gram.
Ammonia (strong)	100 cubic centimeters.
Talcum	5 grams.
Distilled water	100 cubic centimeters.

The purinometer consists chiefly of a graduated cylinder divided into two equal parts by a perforated cock.

The mixed urine for the twenty-four hours is freed of albumin and, with closed cock, 90 cubic centimeters of urine

1 [Magnesia chlorid (crystals), 100 grams; water, 1000 cubic centimeters; ammonia, *q. s.* to give the solution a strong ammonical odor; ammonium chlorid, *q. s.* to dissolve the precipitate which forms.]

and 20 cubic centimeters of Solution I are poured into the tube. The phosphates are at once thrown down, and the cock is then opened. In ten minutes the phosphates have subsided into the lower part of the tube, when the cock is again closed and Solution II added to the mark 100. The precipitate which now forms is a mixture of silver chlorid and silver-purin. The former substance dissolves in the excess of the ammonia present. Shaking the purinometer hastens this process. If the yellow silver-purin contains a few white flocculi, a few drops of ammonia are added. The purinometer is then placed in a closet

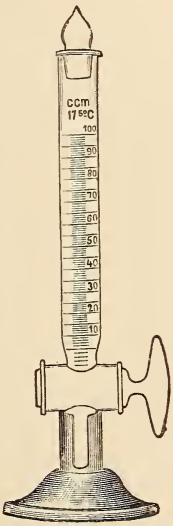


Fig. 104*a*.—Purinometer.

protected from light. The whole procedure takes no more than ten minutes. After one hour all the precipitate is thrown down, but it is better to wait for twenty-four hours before the result is read off.

The amount of precipitate in cubic centimeters multiplied by 1.5 (percentage) and an empiric value (about 0.0010, which is especially determined for each apparatus) gives the percentage of nitrogen. If this value is multiplied by the daily amount of urine expressed in cubic centimeters and divided by 100, the total daily amount of purin-nitrogen is obtained.

EXAMPLE.—The precipitate amounts to 22 cubic centimeters.

$22 \times 1.5 \times 0.0011 = 0.0363$ per cent. purin-nitrogen.

0.0363 per cent. $\times$ 1000 cubic centimeters (total twenty-four hours' urine) $=$ 0.363 (total purin-nitrogen).

The nitrogen factors in the precipitate are to be found in cubic centimeters in the table which accompanies each purinometer.

The following table shows the nitrogen percentage yielded by each cubic centimeter of the precipitate. Multiply this factor by the number of cubic centimeters contained in the twenty-four hours' urine and then by 100.

	Percentage of Purin-nitrogen.
4 cubic centimeters	0.0078
5 cubic centimeters	0.0097
6 cubic centimeters	0.0117
7 cubic centimeters	0.0136
8 cubic centimeters	0.0156
9 cubic centimeters	0.0175
10 cubic centimeters	0.0185
11 cubic centimeters	0.0195
12 cubic centimeters	0.0205
13 cubic centimeters	0.0218
14 cubic centimeters	0.0225
15 cubic centimeters	0.0234
16 cubic centimeters	0.0249
17 cubic centimeters	0.0260
18 cubic centimeters	0.0265
19 cubic centimeters	0.0270
20 cubic centimeters	0.0275
21 cubic centimeters	0.0283
22 cubic centimeters	0.0286
23 cubic centimeters	0.0299
24 cubic centimeters	0.0312
25 cubic centimeters	0.0325

The most accurate results are obtained when the specific gravity of the urine is about 1.015 to 1.025. Above and below these limits deviations occur, but the differences can readily be estimated. When the precipitate is less than 10 cubic centimeters, the number of cubic centimeters must be multiplied by 0.0015; when over 10 cubic centimeters by 0.0012, and when over 22 cubic centimeters by 0.0010.

During the course of the examination neither tea nor coffee should be drunk. For the determination of the amount of endogenous purin, a diet of eggs, milk, cheese, rice, potatoes, bread, and butter should be ordered; for the amount of exogenous purin any quantity of meat may be employed and the purin content determined by the table.

It is clear that the old methods of estimating the excretion of uric acid must be replaced by new determinations of the *total* excretion of purin, and that it is unnecessary to seek new procedures for the determination of uric acid alone.—Brooks.]

Creatinin.—A few drops of freshly prepared dilute sodium nitroprussid solution and some weak sodium hydrate are added to a fresh sample of urine. The ruby-red color at first present usually soon becomes straw-yellow. On addition of acetic acid and boiling a *bluish-green* color appears.

Hippuric acid is best demonstrated by precipitation of the crystals described below.

Of the inorganic constituents, **sodium chlorid** deserves especial consideration. It is most conveniently demonstrated by addition, a drop at a time, of a solution of silver nitrate to the urine acidulated with nitric acid. Chlorid of silver is precipitated in dense, milky-white flocculent and shredlike coagula.

Pronounced **diminution of the chlorids**, which is observed in acute febrile diseases, particularly croupous pneumonia, is manifested by the occurrence of only a faint cloudiness, while an **increase of chlorids**, as occurs especially in the absorption of large exudates, is characterized by an unusually intense precipitation of silver chlorids. [The modification in the amount of chlorids has been attributed to the diet. This view is unquestionably incorrect, to some extent at least, since it is contradicted by the abundant discharge of these substances at the period of convalescence. It is also known that, if chlorids are administered to pneumonia patients, these substances accumulate in the organism and are eliminated only at the time of the crisis (Roger). Likewise the phosphates and sulphates increase when convalescence is established, although to a less notable degree.—Brooks.]

Phosphates of Lime and Magnesia.—1. On heating urine to which a few drops of potassium hydrate have been added, the

"earthy phosphates" are thrown down as a slightly flocculent, whitish precipitate. In faintly acid or alkaline urine the addition of potassium hydrate is unnecessary.

2. On addition of silver nitrate solution the phosphoric acid of the phosphates is precipitated as whitish, flocculent silver phosphate.

3. Addition of ammoniacal magnesian solution to a sample of urine causes a precipitate of triple phosphate, which is characterized by the so-called "coffin-lid" crystals (see below).

Sulphuric acid occurs as a sulphate (so-called preformed sulphuric acid) or as ("combined") aromatic ethereal sulphates. The first is derived principally from the albumin. The "combined" is dependent upon the amount of aromatic bodies originated during proteid putrefaction, which unite with all the available sulphuric acid of the body and appear in the urine as aromatic ethereal sulphates. An increase in these is observed the greater the amount of phenol and indican—in obstinate obstruction, ileus, etc.—present in the urine; their augmented excretion indicates increased protein destruction, especially in fever (Baumann, Kast, and others).

Demonstration.—1. If the urine is first treated with acetic or hydrochloric acid and then with barium chlorid, a whitish precipitate of barium sulphate is formed, which is insoluble in acid.

2. The ethereal sulphates combined with phenol, cresol, indoxyl, and skatol can be demonstrated most conveniently as follows: The urine slightly acidulated with acetic or hydrochloric acid is treated with a large amount of barium chlorid and filtered. After free addition of concentrated hydrochloric acid the *filtrate* is boiled for from twenty to thirty minutes, until barium sulphate is precipitated from the *decomposed* ethereal sulphates. The amount of this precipitate indicates the quantity of ethereal sulphates.

2. CHEMIC DETECTION OF PATHOLOGIC CONSTITUENTS.

The normal acid reaction of the urine, which is faintly acid or alkaline only under the exceptional circumstances already mentioned, is frequently or constantly alkaline in many diseases. This is true especially of the *ammoniacal fermentation* shown by pathologic urine, even on voiding or shortly thereafter. To prove

that the strong alkaline reaction of the urine in such cases is not due to "fixed" alkalies, but to ammonium carbonate, all that is necessary is to hold over the urine a glass rod moistened with hydrochloric acid, when the characteristic sal ammoniac fumes are developed, which otherwise are absent.

Occurrence of Albumin in the Urine (Albuminuria).

In addition to serum-albumin, the most important form, and serum-globulin, which usually accompanies it, propepton (hemialbumose) and pepton, fibrin, hemoglobin, and mucin are met with in the urine.

The *amount of albumin* excreted varies within wide limits. We speak of slight, moderate, and severe albuminuria, according as the daily quantity amounts to 0.1, 0.5, or 1.0 gram or more per mille.

In the great majority of cases every *persistent* albuminuria is an indication of disease of the kidney and less often of the urinary tract. *Transitory* excretion of albumin without renal lesion occurs in fever, venous congestion, nervous disturbances (delirium tremens, epilepsy, cerebral concussion), etc.; also, in a number of chronic constitutional and infectious diseases (severe blood affections, diabetes mellitus, tuberculosis, etc.); finally, as a result of obstruction to the flow of urine due to pressure of stones, neoplasms, etc., upon the ureter.

The designation **physiologic albuminuria** is employed to indicate an occasionally rapidly transitory excretion of albumin, seldom lasting for months or years, often periodically intermittent and always slight (just demonstrable), in which the most careful microscopic examination of the urine reveals not the slightest deviations and in which clinical signs of an acute or chronic disease of the kidneys are absent. This form is sometimes observed in the absence of any antecedent cause (among others, in the newborn); more frequently, however, only after severe bodily exertion, very hearty meals, cold baths, mental strain, violent emotional excitement, etc. For example, among 119 healthy soldiers, Leube found unquestionable albuminuria in 19—*i.e.*, in 16 per cent.—after long marches.

If the albuminuria occurs *periodically*, a definitely regular cycle is often unmistakable. Such cases are designated as

"cyclic albuminuria" (Pavy). Usually the victims are *youthful* individuals in whom the excretion of albumin is induced by change from the recumbent to the upright position. Indeed, the albuminuria is generally most intense very soon after rising or shortly after long marches. *Rest* causes it to disappear entirely. The decidedly pronounced cyclic character of the excretion occurring under ordinary modes of life can at any time be made wholly to disappear by several days' rest in bed. *For the exact observations of this form of albuminuria, classed as physiologic* (Pavy, E. Wagner, Leyden, and others), *it is necessary to make examinations of the urine regularly every one or two hours, and to note the course of the albuminuria under ordinary and variously altered modes of life* (body movements, rest in bed, mental activity, etc.).

As to the justification for assuming the existence of physiologic albuminuria and of this subordinate form, opinion is divided. The author would class himself with those who, from a practical standpoint, view physiologic as well as cyclic albuminuria with much skepticism. Not that he would deny the occurrence of physiologic excretion of albumin, for this has been positively demonstrated by reliable physicians (Leube, Grainger-Stewart, and others), and he himself has made this diagnosis in three cases under observation for a long period. But he considers it extremely difficult to make a positive diagnosis of physiologic albuminuria in individual cases. In fact, many examples of physiologic albuminuria have subsequently proved to be cases of Bright's disease. It must also be remembered that many cases of contracted kidney at times show no trace of formed elements and that the other clinical phenomena may be obscured. In such instances consideration of the specific gravity will, under certain conditions, decide the question. Senator, however, justly warns against declaring as physiologic even an insignificant cyclic albuminuria occurring in persons at or beyond middle age; in young individuals he considers *an albumin content of 0.4 to 0.5 per mille as the limit beyond which an albuminuria can no longer be recognized as physiologic!* If the quantitative estimation is made with the Esbach method, especial care must be observed (see page 302).

Qualitative Tests for Albumin.

The urine to be examined must be pure and free from extraneous admixtures. *It is also advisable to test the urine voided at different times of the day.* The urine formed at night generally gives the least information of existing disturbances; the urine voided during the day, especially after the first meal, is to be preferred. Cloudy or highly concentrated urine should always be *filtered* before testing.

Demonstration of serum-albumin, which is usually accompanied by serum-globulin:—

1. Heller's Nitric Acid Test.—Equal parts of urine and pure nitric acid are so placed in a test-tube that the urine, by careful addition, forms a layer *above* the acid without admixture. In the presence of albumin (albumose and mucin) there is formed at the point of contact a distinctly outlined, white ring which, when small amounts of albumin are present, can sometimes be recognized only after several minutes and occasionally only when the test-tube is held against a dark background.

It is best to dilute highly concentrated urine rich in urates with water, otherwise precipitations with nitric acid may be produced; these precipitates, however, can be distinguished from albumin by their color as well as the *higher position* of the ring and also *by their disappearance on application of gentle heat.*

An opalescence may also occur after use of turpentine and balsam of copaiba and Tolu (Sommerbrodt's capsules). This, however, is dissolved by shaking with alcohol. The usually faint *ring* produced by *mucin* generally disappears on shaking. If these precautions are observed, the test is *extremely* reliable. It is also very delicate, since 0.02 per mille of albumin is distinctly shown by it.

[A very handy and useful instrument for the detection of small quantities of albumin in the urine is the **albumoscope** (horismascope), shown in the accompanying illustration (Fig. 105). It obviates the use of the pipette. The urine is first placed in the larger cylinder, and nitric acid then poured into the funnel of the smaller tube until the level of the acid reaches about the center of the black background in the larger tube. By looking through the fluid against the black surface, faint traces of

albumin can readily be made out. When the albumin is very small in amount, it is best to set the instrument aside for from fifteen to twenty minutes before inspection.—Brooks.]

2. Nitric Acid and Heat Test.—The simple heat test suffices as an albumin reagent only in such urines as have a distinctly acid reaction, because, in faintly acid or alkaline urine, earthy phosphates, as well as the albumin, is precipitated by boiling. It is, therefore, advisable first to add nitric acid, which is preferable to acetic acid, because the latter, even in slight excess, dissolves slight amounts of albumin. For this reason an amount of nitric acid equal to about one-fifth of the bulk of the urine is added and the mixture heated to boiling. The white precipitate

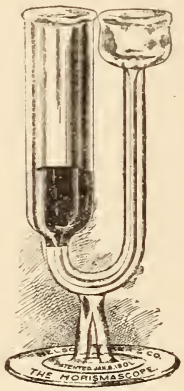


Fig. 105.—Albumoscope.

which *at once* begins to form, and which is usually brownish if blood is present, indicates the presence of albumin. The fine, sandlike precipitate observed after a time in highly concentrated urine must not be mistaken for albumin. The test is quite delicate, but it is excelled by others in certainty and convenience.

3. Acetic Acid-Potassium Ferrocyanid Test.—The urine is first strongly acidulated with acetic acid and then 5- to 10-per-cent. potassium ferrocyanid solution *carefully added drop by drop.* In the presence of albumin a dense, white precipitate generally appears at once, or, if slight amounts are present, a distinct cloudiness is seen only after several minutes. If a cloudy precipitate appears at the time of acidulation (due to precipitation

of mucin), it is necessary to *filter* the urine; it is best first to dilute very concentrated urines. The reaction is *extremely delicate and certain,* and, since boiling is omitted, it is very convenient. *For office work it unquestionably deserves to be given the preference.*

4. Test with Acetic Acid and Concentrated Common Salt or Sodium Sulphate Solution.—The urine is first treated with acetic acid and then with saturated solution of common salt or sodium sulphate and boiled. The precipitate, which is *at once* formed, is a very positive indication of the presence of albumin. When *large amounts* of albumin (and albumoses) are present, a precipitate appears even before heat is applied. Heating dissolves the albumoses; faint traces of albumin can then be recognized.

5. Test with Metaphosphoric Acid.—This test, first recommended by Hindenlang, possesses the following advantages: The physician can easily carry the reagent about with him, and an examination for albumin can quickly be made. A small piece of the white stick, deliquescent in the air, is placed in a portion of the urine to be examined and then shaken. A distinct white precipitate indicates the presence of albumin. A saturated solution, added a drop at a time, also produces a precipitate of albumin (according to Hoppe-Seyler, pepton is not precipitated). The test is very convenient, but is less delicate and positive than the methods first mentioned. It should, therefore, be used only as a means of orientation. Trichloracetic acid crystals may be recommended for the same purpose.

6. Picric Acid Test.—As much of the crystals as can be held upon the point of a penknife is added to the urine and shaken. A distinct yellow flocculent precipitate is produced in the presence of albumin. The test is more marked when made with a saturated (aqueous) solution, added a drop at a time. The test is reliable, delicate, and convenient. It may be remarked that, according to Jaffe, creatinin is also precipitated by picric acid. The yellow color often disturbs the test.

7. Potassium Sulphocyanate-Acetic Acid Test (Zouchlos).—The reagent consists of 100 parts of 10-per-cent. potassium ferrocyanate solution and 20 parts of acetic acid. When added a drop at a time, it produces a distinct cloudiness in the presence of albumin. The test is almost as delicate and equally as con-

venient as No. 3, and, according to von Jaksch, indicates 0.007 per cent. of albumin.

8. Spiegler's Test.—To a sample of urine which has been freed of mucin by strong acidulation with acetic acid are carefully added a few drops of the following freshly prepared reagent: Hydrargyri bichloridi corrosivi, 8; acidi tartarici, 4; glycerini, 20; aquæ destillatæ, 200. In the presence of minute amounts of albumin a whitish ring appears.

Geissler's albumin reagent paper and **Stütz's albumin reagent capsules** possess no advantages so far as convenience is concerned. On the other hand, both are highly unreliable. The first test is based upon the fact that a precipitate is produced in albuminous urine by solutions of citric acid and iodosublimate. The capsules contain citric acid, sodium chlorid, and corrosive sublimate, and when added to urine also produce precipitation of albumin.

For the detection of the minute traces of albumin present even in *normal* urine (in the latter instance very probably derived from the vessels of the glomeruli), Posner recommends the following: A precipitate is formed by adding to the urine three times its volume of alcohol or concentrated watery solution of tannin. The precipitate is then washed in water and dissolved in acetic acid. The tests Nos. 3, 4, and 7 then show traces of albumin.

Detection of Globulin.—To demonstrate the presence of globulin, which is usually associated with serum-albumin, the following method is convenient: About from 30 to 50 cubic centimeters of urine are filtered and diluted with ten times its volume of distilled water. If a cloudiness or flocculent precipitate is gradually produced on addition of dilute acetic or boric acid, then more or less globulin is present.

Detection of Propepton (Hemialbumose).—If Heller's nitric acid test or the acetic acid-potassium ferrocyanid test produces a distinct precipitate which disappears on heating and reappears on cooling, this is indicative of the presence of albumose.

If in addition to albumoses albumin also is present, the latter must be precipitated by careful addition of acetic acid to boiling urine and removed by filtration. The filtrate is rendered strongly alkaline with potassium hydrate, and 10-per-

cent. copper sulphate added *drop by drop*. The red or bluish-red color *(biuret reaction)* indicates the presence of albumose.

Albumoses occur but seldom in the urine, and aside from certain diseases their presence is transitory.

[Boston's Method for Bence-Jones Albumose. — From 15 to 20 cubic centimeters of the filtered urine are placed in a test-tube, and to it an equal quantity of a saturated solution of sodium chlorid is added, and the tube shaken to effect a perfect mixture. Boston's directions are as follows:—

"Two or three cubic centimeters of a 30-per-cent. solution of caustic soda are now added, shaking vigorously.

"The upper one-fourth of this column of liquid is gradually heated over a flame to the boiling-point, when a solution of lead acetate (10 per cent.) is added, drop by drop, boiling the upper previously heated stratum of liquid after each additional drop.

"When the drop of lead acetate solution comes in contact with the liquid a copious pearly or creamy cloud appears at the surface, which becomes less dense as the boiling-point is neared, and when ebullition is prolonged for from one-half to one minute the upper stratum shows a slight browning, which deepens to a dull black. The lower portion of the heated liquid shows less marked blacking, and below this point for some distance there is seen a variable degree of browning. Standing intensifies the reaction; but if this be prolonged for several hours the black precipitate falls through the clear stratum of liquid, collecting as a coarsely granular pigment in the bottom of the tube."—Brooks.]

Detection of Pepton.—Our knowledge of peptonuria is due to Maixner, von Jaksch, and others. It appears *independently* of albuminuria, and occurs *chiefly on entrance into the blood of pepton derived from disintegrated leucocytes and pus-corpuscles.*

Peptonuria is most frequently observed as a *pyogenic form* in suppurative pleural exudates and other suppurations within the body, as well as in croupous pneumonia at the time of resolution: *i.e.,* when the conditions for the disintegration of the leucocytes and the absorption of their products are particularly favorable. The demonstration of pepton in the urine does not, in every instance, justify the conclusion that internal suppuration

and the like exist. Aside from the occurrence of pepton in the resolution stage of pneumonia, which has already been referred to, it is also observed in healthy puerperal women. Nevertheless the demonstration of pepton may be of diagnostic value in many instances. For example, according to von Jaksch, the *absence* of peptonuria in the presence of meningitic phenomena may be decisive for diagnosis of the tubercular form.

For the *detection* of pepton the urine is first rendered absolutely free of albumin. About 50 cubic centimeters of concentrated sodium acetate solution are added to 500 cubic centimeters of urine, and then as many drops of concentrated solution of iron chlorid as will give the fluid a decidedly reddish color. Then sodium or potassium hydrate is carefully added, drop by drop, until the acid reaction is neutralized or the fluid just reddens blue litmus-paper. The fluid is then boiled, and, when cool, filtered. The filtrate, which must not give the slightest cloudiness with acetic acid and potassium ferrocyanid, is now tested with the above-mentioned biuret test. If pepton is present a distinct red or violet color is produced (Hofmeister).

Detection of Fibrin.—Fibrin coagula are but seldom found in the urine. For detection, they are separated by filtration and washed repeatedly in 5-per-cent. solution of sodium chlorid until the washings no longer give the albumin test. If the residue upon the filter is now treated with 1-per-cent. soda solution and boiled, complete solution occurs. Application of the Heller or the acetic acid-potassium ferrocyanid test after the solution has cooled will now show the albumin reaction.

Detection of Mucin.—In many diseases of the kidneys, and especially of the urinary bladder, the amount of mucin is often considerably increased. It is very probable that in this instance we have to deal with nucleo-albumin, which will be spoken of later.

To *determine its presence* the urine is first diluted with water, filtered, and acetic acid in excess added to the filtrate. In the presence of mucus a distinct precipitate is formed, which disappears on addition of potassium hydrate, but reappears on further addition of acetic acid.

Quantitative Estimation of Albumin.

For the **quantitative estimation** of the **amount of albumin** excreted in twenty-four hours, if it is not desired to make an

Fig. 106.—Esbach's Albuminometer.

Fig. 107.—Improved Esbach Albuminometer.

exact analysis, the test with *Esbach's albuminometer* suffices (Figs. 106 and 107). This simple and cheap instrument consists of a test-tube upon which are etched the marks *R* and *U*, and a finely graduated scale for distinctly determining the height of the column of precipitated albumin.

It is absolutely essential to observe the following points:—

1. The urine must have an *acid* reaction. Neutral and alkaline urines are, therefore, to be acidulated with acetic acid.

2. The density of the urine must not exceed 1.006 or 1.008; it must, therefore, be diluted correspondingly.

3. The test must always be made at *room temperature,* since differences of temperature influence decidedly the height of the precipitate.

The following reagent is employed: 10 grams of pure picric acid and 20 grams of air-dried, chemically pure citric acid are dissolved in 800 cubic centimeters of water and the total volume brought to 1000 cubic centimeters by addition of water at 15°C. [60° F.].

In performing the test the Esbach cylinder is filled with urine to the mark *U* and the reagent added to the mark *R*. The tube is then closed with a rubber cork and inverted slowly about fifteen times. The tube is then allowed to stand at rest for twenty-four hours at as uniform a temperature as possible. At the end of this time *the height of the precipitate, as shown by the graduated scale, gives the number of grams of albumin contained in one liter of the urine examined.*

The method is attended by many sources of error, and is adapted only for approximate *estimation* of the albumin loss. In this respect, it is, however, far inferior to the formerly employed method of determination of the "volumetric percentage," which was based upon the estimates of the amount of albumin from the height of precipitate obtained in a test-tube with the nitric acid-heat test. If, however, the determinations are conducted with strict observation of the above-mentioned points, quite accurate information in regard to the increase and decrease of the albumin excretion will be obtained. The method is, therefore, of decided value, even in the diagnosis of "physiologic and cyclic albuminuria."

The **polarization** method of estimating the amount of albumin excreted is based upon the fact that all albumin bodies turn the plane of polarized light to the *left*. The test can readily and easily be made, but it also gives only approximate values, because even nonalbuminous urine also turns the plane of light slightly to the left (see page 321).

Lipuria.

Fat sometimes occurs in the urine in droplets, sometimes in crystalline form. It is seldom observed, and then almost only in pregnant women, in cases of "large, white kidney," and in phosphorus poisoning. The whitish-yellow, cloudy urine clears on shaking with ether. Fat occurs somewhat more frequently in the urine of persons suffering from **chyluria**, who excrete a *fatty* and *albuminous* urine. This presents a distinctly milky appearance and not rarely contains loosely arranged, whitish or transparent gelatinous coagula (fibrin). The whole of the urine may occasionally coagulate into a very spongy mass, which assumes the form of the vessel containing it.

The urine loses the milky appearance on addition of some sodium hydrate and shaking with ether. The "emulsified" fat is dissolved, but perfect clearing is usually not obtained. In addition to the very variable *amount of fat,* from 0.5 to 2 per cent. or more of *albumin* is always present. If, as is not infrequently the case, the urine also contains *blood (hematochyluria),* it has a cherry-red color, and assumes the yellowish-white, milklike character only after the blood has settled to the bottom. The urine just described is observed almost exclusively in inhabitants of the tropics (especially in China, Japan, Egypt, Brazil, etc.), or such who formerly lived there (see page 114). Brieger and others have observed chyluria in Europeans who had never been in the tropics. These, however, are extremely rare exceptions.

Hematuria and Hemoglobinuria.

Bloody-red urine contains either pure blood from the kidneys and urinary passages or dissolved and otherwise altered blood-coloring matter. In the first instance the condition is known as *hematuria;* in the second as *hemoglobinuria* or *methemoglobinuria.*

In hematuria the urine is bright or dark red, distinctly bloody, dichroic, and sometimes contains broad, somewhat ragged blood-clumps (vesical bleeding) or earthwormlike blood coagula (renal pelvic bleeding), which are voided as such, or coagulation does not occur until after urination.

Usually positive determination of the seat of the hemorrhage can be made only with the microscope. Hematuria occurs in gonorrhea, acute cystitis, calculi and ulcerations in the bladder and in the pelvis of the kidney, and in tuberculosis and neoplasms of the urinary apparatus.

Chemic Detection of Blood-Coloring Matter.

1. Heller's Test.—The urine is rendered strongly alkaline with potassium hydrate and boiled. On cooling, the blood-coloring matter is carried down with the precipitated earthy phosphates and tinges the latter (which otherwise appear as white flocculi) brownish or garnet-red.

2. Almen's Test.—Equal volumes of fresh tincture of guaiac and old oil of turpentine are placed in a test-tube and shaken until an emulsion is formed. To this the urine is carefully added. In the presence of blood there at first appears a bluish-green, sometimes light- or dark- blue ring, and, on shaking, the whole mixture assumes a diffuse blue color. The urine to be tested must be distinctly *acid,* and if alkaline in reaction it must be acidulated with acetic acid. The presence of pus without blood gives the same reaction with this test.

While the diagnosis of hematuria can often be made with almost absolute certainty from the appearance of the urine and the chemic examination, the diagnosis of hemoglobinuria, which occurs as a result of hemoglobinemia *(q. v.),* can usually be determined only by microscopic and spectroscopic analysis (see below).

Bile-Coloring Matters.

These occur in the urine as *bilirubin,* the oxidation products of which are *bilivirdin, bilifuscin,* and *biliprasin,* or as *urobilin s. hydrobilirubin,* which is formed by reduction from bile- and blood- coloring matter. Urine containing bile-coloring matters appears light- or dark- beer brown, and on shaking shows a yellow or yellowish-green foam.

Detection of Bilirubin.—1. Chloroform Test.—About 10 drops of chloroform are added to half a test-tubeful of urine, and the solution thoroughly shaken. The finely divided chloroform brings down the coloring matter, and appears as a dense, canary-

yellow precipitate. If the chloroform extract is now mixed with ozonated oil of turpentine and some dilute potassium hydrate, a distinct greenish color will be observed in watery solution.

2. Gmelin's Test.—A few cubic centimeters of pure nitric acid, to which 1 or 2 drops of fuming acid have been added, are carefully overlaid with urine by means of a pipette. At the point of contact there forms a green, blue, violet, or reddish-yellow ring. Only the *green* ring is positive. Blue and red colors may be produced by indican or urobilin.

3. Gmelin-Rosenbach Filter Test.—After the urine has been filtered through a small paper filter, whereby the latter is colored a decided yellow, the inner surface of the filter-paper is touched with the above-mentioned nitric acid mixture. In the presence of bile-coloring matter a distinct play of colors from green to red will sometimes be observed. This test is extremely delicate and highly to be recommended.

4. Rosenbach has proposed the use of 5-per-cent. solution of chromic acid instead of nitric acid, with which a pure green color exclusively is obtained. Only 1 drop, however, must be carefully added. The filter test is also well adapted here.

Detection of Urobilin.—Two to 5 drops of a 10-per-cent. solution of zinc chlorid are mixed with the urine and then ammonia added until the precipitated zinc oxid is redissolved. If a greenish fluorescence can be detected in the filtered solution when held against a dark background, the presence of urobilin is demonstrated (Fr. Müller).

Bilirubin is excreted in the urine in every case of icterus after absorption of bile into the blood when the flow of bile into the intestine is obstructed by any cause.

Urobilin is not connected with icterus *per se*. On the contrary, it is formed in the intestine by the action of putrefactive bacteria by reduction of bilirubin. It is, therefore, usually absent when the flow of bile into the intestine is suspended as the result of chronic occlusion of the ductus choledochus by neoplasms, gall-stones, and the like. This view, which is advocated particularly by Fr. Müller, is supported by the fact that the intestine and urine of the *newborn,* in whom the action of putrefactive bacteria is out of the question, are always found to be free from urobilin, and, furthermore, that, after re-estab-

lishment of the flow of bile into the intestine, very large amounts of hydrobilirubin at once appear. In addition to its occurrence in icterus it is observed especially in urine of febrile patients. It is often formed in voided urine from urobilinogen on exposure to the air.

Biliary Acids.—In addition to the bile-coloring matters, biliary acids also occur in the urine (icterus).

Detection by Pettenkofer's Test.—A few drops of urine, to which a grain of cane-sugar has been added, are evaporated with a drop of concentrated sulphuric acid upon a porcelain dish, by aid of gentle heat. In the presence of biliary acids a distinct *purple color* is produced which may change to a purplish violet. (As similar reactions are occasionally caused by other substances, a more complicated method for the elimination of indican, albumin, fat, etc., is necessary for exact determinations. For directions for such a procedure the reader is referred to larger text-books.)

Indicanuria.

Indican, which is a product of proteid putrefaction in the intestine and is always present even in normal urine, may be greatly increased in pathologic processes in the gastro-intestinal canal, especially in those which give rise to decided proteid putrefaction in the intestine (ileus, strangulation, etc.), as well as in putrid suppurations in other parts of the body. Although the excretion of indican is very variable even in such disturbances, a very strong reaction permits the assumption of the existence of abnormally intense proteid decomposition in the intestine or other parts of the body.

In the latter process *indol* is first formed. After absorption the indol is oxidized into indoxyl, and, combined with the sulphuric acid of the urine, is excreted as indoxyl-potassium sulphate.

[In this connection it may be stated that it now appears to be quite well established that gastro-intestinal disturbances characterized by excessive fermentation and putrefaction induce degenerative arterial changes. There is ample foundation for the belief that the influence of syphilis in the production of scleroses has been exaggerated, and that many arteriosclerotic

conditions attributed to this source are in reality due to the prolonged continuous influence of toxins absorbed from the intestinal tract. Indeed, Heubner and Lancereaux deny to syphilis all influence in the production of arteriosclerosis (Roger). The process in intestinal disturbances of the type above mentioned is a true intoxication of the organism. Although the liver is usually the organ most markedly involved (sclerosis), the toxins sooner or later pass the barrier opposed by this gland and act upon other organs, especially the arteries of the principal emunctories, namely: the kidneys. In the latter organs the parenchyma (the epithelial cells) is gradually and insidiously destroyed and equally slowly and insidiously succeeded by fibrous tissue. Individuals suffering from this disturbance usually pass a urine but slightly deviating from the normal as regards specific gravity, color, and amount. Chemically, however, large amounts of indican and occasionally traces of albumin are found. Microscopic examination shows occasionally small hyaline and delicate, granular casts; rarely epitheliated casts; very frequently calcium oxalate; and often uric acid: indications of suboxidation and disturbance in general metabolism. Many such cases are diagnosticated as "gouty" or "rheumatic" and treated accordingly.—Brooks.]

Detection of Indican by Jaffe's Test.—Free the urine of various substances interfering with the test by the addition of one-fourth its volume of 10-per-cent. solution of lead acetate, and then filtrating. To the filtrate add an equal amount of concentrated hydrochloric acid (splitting) and 1 to 2 drops of a saturated solution of calcium chlorid diluted one-half with water, or 3 or 4 drops of 0.5-per-cent. solution potassium permanganate (oxidation). On further addition of this solution there appears, when indican is present, first a bluish-green tint, later a distinct blue color. If a little chloroform be now added and the whole thoroughly shaken, the indigo is thrown down as a blue precipitate.

The amount of indican normally present in the urine gives with this test only a rose or faint-violet color.

In rare instances a deep *black coloration* of the urine is induced by **indican**; so that confusion with melanin, which will next be considered, may occur.

In such cases the urine at the time of voiding is, as in genuine melanosis, only dark reddish or brownish in color, and becomes blackish only on standing, or on boiling and addition of nitric acid. The dark coloration can also persist or be intensified by addition of chromic acid, sulphuric acid, chloroform, and with Jaffe's indigo test. If, however, the indican is precipitated by milk of lime and the black color no longer remains, then the cause of the latter is indicanuria (Senator).

In rare instances *indigo* (**urine-blue**, Virchow) appears as such in the urine and may then either color all of the urine blue or, what is relatively more frequent, sink to the bottom as blue flakes which show the delicate, indigo-blue needles arranged in stellate form. The urine is usually voided clear and pale, and presents the blue color only after some time (Virchow). A deep-blue urine may, however, be freshly voided (Litten). In such cases *nonpigmented* carcinoma of the stomach and liver is usually present.

Melanuria.

In **melanuria**, also, the urine is pale yellow, yellowish brown, and perfectly clear, and becomes deep black and opaque only on standing or after addition of oxidizing agents. It occasionally presents an inky appearance even when voided. In the first instance the coloring matter is excreted as *melanogen*, in the second as *melanin*. Melanogen is a colorless chromogen which becomes deep black only on oxidation. Bromin-water, chromic acid, nitric acid, and iron chlorid, among others, at once cause the *melanin* to develop. Confusion with indicanuria can be avoided by previous precipitation of indican (see page 306).

Genuine melanuria is of high semeiotic significance in the diagnosis of melanotic tumors located in internal organs, particularly in the liver. Exceptions are so rare (Litten, Senator) that they scarcely need be considered in diagnosis.

Alkaptonuria.

The usually rare **alkaptonuria** is characterized by the fact that the urine on standing exposed to the air, and still more on occurrence of ammoniacal fermentation, assumes a *brown* or even *blackish* color. Trommer's sugar test (see page 315) gives

a positive reaction, but, on the other hand, Nylander's (bismuth) test does not. The urine remains optically inactive, and reduces ammoniacal silver solution in the cold. By these features it can be distinguished from diabetic urine.

Whether alkapton, which occurs without disturbance of health and *is often observed in brothers and sisters,* is caused by abnormal fermentation in the intestinal canal (Baumand and Wolkow) has not been determined. The fact that alkapton has never been found in the stools even after free administration of cathartics speaks against such an assumption. Alkapton may be considered as a derivative of tyrosin. Embden found that the excretion of uric acid was decidedly diminished in subjects of alkaptonuria.

Pentosuria.

In **pentosuria,** also, the urine is characterized by strong reducing properties. *Trommer's, Fehling's,* and *Nylander's* tests give positive reactions; there is, however, only slight power of deflection of polarized light, and the fermentation test gives negative results. Tollen's reaction is characteristic of pentoses.

To 5 or 6 cubic centimeters of fuming hydrochloric acid is added sufficient phloroglucin to leave some undissolved. The solution is now divided into two equal parts, and to one half is added half a cubic centimeter of the urine to be examined and to the other half the same amount of normal urine. If the samples be now heated in a glass with boiling water, the pentose urine quickly assumes a *brilliant-red* color, which extends from above downward, while the control urine remains unaltered. (It is advisable previously to decolor both samples of urine with animal charcoal.)

The following test is also reliable and convenient: As much orcein as can be held upon the point of a penknife and 5 cubic centimeters of hydrochloric acid (spec. grav., 1.19) are added to 5 cubic centimeters of urine in a test-tube and heated to boiling. In the presence of pentose a distinct *bluish-green* coloration appears. The coloring matter may be extracted by shaking with amyl alcohol, and when viewed with the spectroscope shows an absorption band in red (Blumenthal).

ALTERATIONS IN APPEARANCE AND CHEMIC CHANGES PRODUCED IN THE URINE BY THE ENTRANCE OF CERTAIN DRUGS INTO THE SYSTEM.

1. By administration of **rhubarb** and **senna** the urine is colored intensely yellow, owing to the presence of chrysophanic acid. If such a urine is treated with potassium hydrate, a *brilliant-red* color develops, which disappears on addition of acid (see "Santonin").

2. After **santonin** a similar yellow color is observed which is transformed into a *rose-red* on addition of potassium hydrate. If ether is shaken with the urine, the ether remains colorless, while on shaking with rhubarb or senna urine it becomes yellow, and addition of potassium hydrate to the yellow-colored ethereal extract causes a distinct red coloration at the point of contact (Penzoldt).

3. Urine containing **tannin** is colored *grayish green* to *blackish blue* on addition of dilute solution of iron chlorid.

4. Urine containing **balsam of copaiba** occasionally gives, on boiling and addition of hydrochloric acid, a distinct cloudiness, which, unlike albumin precipitate, is dissolved by alcohol. Addition of hydrochloric acid colors the urine a beautiful *red,* and when heated at the same time it assumes a *violet* hue.

5. The light to dark blood-red urine occurring after free administration of **antipyrin,** and which not infrequently manifests dichroism, becomes a deep-*brownish-red* on addition of dilute solution of iron chlorid.

6. After administration of **naphtalin** the urine assumes a dark color. On addition of a few drops of ammonia a *blue fluorescence* is observed.

7. In the presence of **salicylic acid** in the urine the addition of iron chlorid first produces a *yellowish* precipitate of earthy phosphates, and on further addition a bright-*violet-blue* coloration. If very small amounts are to be detected, it is necessary, after previous acidulation of the urine with some sulphuric acid, to add to the urine an equal volume of ether and extract the salicylic acid by shaking. The latter is taken up by the ether, which is then poured off and treated with iron chlorid solution.

8. After the entrance of **carbolic acid** by ingestion, inspiration, or absorption from wound and ulcer surfaces the urine appears *brownish green,* and a still *darker green* on long standing. If a sample of such urine is placed in a test-tube and some bromin-water added, a *bright-yellow* precipitate is produced, in which, after a time, glistening crystals in the form of needles and scales are found.

9. **Potassium iodid** urine treated with a few drops of fuming nitric acid and about one-third its volume of chloroform gives a beautiful rose-violet color to the separated chloroform on shaking. Instead of chloroform, carbon bisulphid can be employed, the odor of which, however, is very repulsive. The following test is still more delicate: A few drops of starch paste are thoroughly mixed with the sample of urine in a test-tube and then some fuming nitric acid added. At the point of union there occurs, even in the presence of but 0.001 per cent. of iodin, a *deep-blue ring,* which, however, is transitory. Or the urine is mixed with an equal volume of concentrated hydrochloric acid in a test-tube and overlaid with 2 or 3 drops of weak chlorin-water. A *brownish-yellow* stratum is formed upon the surface, which becomes *blue* on addition of starch solution (Jolles).

10. **Potassium bromid** is detected by adding chlorin-water to the urine, in order to liberate the bromin, and afterward shaking with chloroform. When the latter settles, it appears *dark yellow* in color, owing to the presence of bromin. Or the urine is treated with chlorin-water and then shaken with ether. The latter is colored *yellow* by the liberated bromin, and, after decanting and addition of potassium hydrate, can again be decolored.

GLYCOSURIA [MELLITURIA] AND DIABETES MELLITUS.

The sugar present in human urine is grape-sugar (glucose). The question whether this substance in small amounts may be considered as a normal constituent of the urine appears to be an unsolved problem even at the present day. As glucose is constantly present in the blood, in amounts of 0.5 to 2 per mille, its appearance in the urine would *a priori* be expected. Up to the present time, however, the opinions of distinguished investigators are at variance in this respect. While Brücke, Meissner, and others claim to have demonstrated the constant occurrence of

sugar in the urine, Maly, Seegen, Külz, and others, upon the basis of exhaustive researches upon a great number of urines, oppose the correctness of such an assumption.

A decision of this question, which is of equal importance in physiology and practical medicine, is rendered difficult by the further researches made with reference to reducing substances and those which deflect the plane of polarized light to the right. *It has been proved that the urine of healthy individuals possesses a distinct power of reduction, chiefly by virtue of the uric acid, creatinin, and the compounds of glycuronic acid which it contains;* that glycuronic acid, which very probably is an intermediate product of metabolism after both meat and carbohydrate diet, manifests dextracircumpolarization; and *that, finally, the excretion of reducing substances is increased by liberal meat diet, and particularly by fever.*

When E. Fischer demonstrated as a characteristic property of phenylhydrazin—discovered by him—that this body forms, with sugar, yellowish, crystalline compounds distinguished by high melting-point,—the so-called azones,—an especially delicate method for the detection of minute quantities of sugar, and one undisturbed by other reducing bodies present in the urine, appeared to have been offered. This hope, however, was shattered when Thierfelder proved that glycuronic acid also formed the same crystalline compounds with phenylhydrazin. However, there appears to be an important point of distinction, in so far as these crystals have a much *lower* melting-point.

With regard to this difference, Moritz found in perfectly healthy individuals the constant formation of phenylglucosazone crystals, which, above all else, were characterized by their high melting-point: between 196° and 206° C. [384° and 402° F.]. With the fermentation method, also, which will subsequently be discussed, Moritz succeeded in obtaining distinct, partly intense results in three instances in six healthy men who, along with full meals, ingested large amounts of sweets, ices, and sekt, while Nylander's test *(q. v.)* gave positive results four times. By this achievement the possibility of transitory alimentary glycosuria (*G. alimentaire* of Claude Bernard), is again demonstrated, and under such circumstances as very often occur in ordinary life. Further investigations in this direction are needed.

Transitory glycosuria is occasionally observed after administration of drugs (*e.g.,* thyroid tablets), and intermittent glycosuria in pancreatic colic. [Also after chloroform and ether anesthesia, after epileptic and hysteric paroxysms, and violent emotional excitement. Under these circumstances it is usually slight in amount.—Brooks.]

In **diabetes mellitus** the clinical features are decidedly different. Here we have to deal with a chronic disease in which more or less large quantities of sugar are constantly excreted, and can usually be readily demonstrated by methods given below. *The organism is no longer capable of consuming the glucose derived from the carbohydrates, and, even upon an exclusive meat diet, possesses the pathologic ability to form sugar* in quantities which could not possibly be derived from the slight amount of carbohydrates of the meat, and which often constantly increases on enlarged meat diet. The admirable experiments of von Mering and Minkowski [and Opie] have brilliantly demonstrated the clinical and pathologico-anatomic observation that the pancreas occasionally plays an important rôle in diabetes. Genuine diabetes invariably occurs after removal of the pancreas.

In diabetes an abnormally pale, clear, and acid urine is voided. The quantity is usually considerably increased, and may vary between 1.5 and 10 liters. The specific gravity is always increased, varying between 1.020 and 1.060. The odor is usually somewhat stale or resembles that of fruit. The amount of sugar excreted may vary from quantities which are just demonstrable up to 10 per cent. It is very decidedly influenced by diet, the consumption of carbohydrates increasing the amount of sugar, and strict meat diet causing it wholly to disappear (*mild* form); or it persists under exclusion of carbohydrates and is increased by augmented meat diet (*grave* form).

Active walking and other bodily exercise, also, usually diminish the excretion of sugar; on the other hand, exhausting bodily exertion (Külz) and emotional excitement may increase it.

In the diagnosis of the so-called "mild form" the fact that the urine contains sugar only at certain times of the day and at other times is free from sugar is of importance. Very often, however, sugar is found when the urine is voided from one-half

to one hour *after the first "roll" breakfast, because the sugar more readily passes into the urine when the carbohydrates are taken upon an empty stomach* (Külz, Worm-Müller). If it is desired to test a portion of the urine, care should be taken that at least the "breakfast" urine is secured. Otherwise it is advisable to examine a sample taken from the *total quantity* voided in twenty-four hours, since in this way information as to the total amount of sugar excreted in twenty-four hours can also be obtained, for, aside from the fact that many single samples may contain much sugar and others may be wholly free from it, the percentage of sugar in the former is very variable. After considerable experience, based upon a large series of examinations, an approximate percentage estimation can be made, provided the total quantity passed in twenty-four hours and the specific gravity are known.

Approximate Percentage Estimation of Sugar. (Naunyn.)

Amounts.	Spec. Grav.	Quantity of Sugar.
1½ liters	1.030	About 1 to 2 per cent.
3 "	1.030	Usually over 5 " "
3 "	1.025	About 3 to 4 " "
6 to 8 "	1.030	Usually above 8 " "

If only slight amounts of sugar are found with the sugar tests now to be described, a diagnosis of diabetes mellitus should be made with caution, and the possibility of the presence of a physiologic or transitory alimentary glycosuria constantly kept in mind. In such cases repeated testing of the urine for sugar is necessary. It should be learned whether or not ingestion of carbohydrates, especially cane-sugar (Külz), rapidly increases the percentage of glucose in the urine.

In many severe cases *albuminuria* coexists. The latter sometimes follows a strictly enforced Cantani meat cure undertaken for the purpose of quickly suppressing the glycosuria. Whenever it exists, it is absolutely necessary to remove the albumin from the urine before the test for sugar is made.

For this purpose the urine is boiled and the beginning cloudiness transformed into a precipitate by careful addition of acetic acid, drop by drop. After boiling for a short time the urine is filtered. If the filtrate is perfectly clear, then all the albumin has been precipitated.

Qualitative and Quantitative Tests for Sugar.

These depend upon the following properties of sugar:—

1. In alkaline solution it reduces various metallic oxids, such as copper and bismuth oxid.

2. It is decomposed in hot solutions by potassium hydrate with the formation of a yellow or reddish-brown precipitate.

3. With phenylhydrazin it forms yellowish, crystalline compounds which are almost insoluble in water: the so-called azones.

4. It is decomposed into alcohol and carbonic acid through the agency of yeast.

5. It turns the plane of polarized light to the right.

Sugar Tests.

1. Trommer's Test.—The urine is rendered alkaline with potassium or sodium hydrate (from one-fourth to one-third its volume), and then, under constant shaking, as much 10-per-cent. copper sulphate solution added, drop by drop, as will be dissolved. The upper portion of the mixture is then boiled until a yellowish-brown precipitate appears. Further development of the precipitate is allowed to take place spontaneously. The reduction now advances into the remaining blue column of liquid. The yellowish-red precipitate is due to cuprous hydroxid and the reddish precipitate to cuprous oxid.

A simple yellow coloration is not decisive, and the same is true of a precipitate occurring after some time.

If a distinct yellowish-red precipitate forms *before* boiling, it is very probable that the urine contains sugar. It must not be forgotten, however, that normal urine contains a number of reducing substances (uric acid, creatinin, glycuronic acid [alkapton]), which, under certain circumstances, may give a confusing reddish coloration, and that, on the other hand, even in the presence of small quantities of sugar the cuprous oxid formed may be held in solution by creatinin and the appearance of the characteristic color thus be prevented (see also "Alkaptonuria" and "Pentosuria").

Sugar in amounts less than 0.5 per cent. is not distinctly shown by this test.

2. Test with Fehling's Solution.[1]—As much of this solution as will remain dissolved is added to the urine, drop by drop. The top of the column of liquid is now heated and the reaction allowed further to develop as described in test No. 1.

[Fehling's solution is made as follows:—

1. Dissolve 34.652 grams of pure crystallized copper sulphate in 200 grams of distilled water.

2. Dissolve 173 grams of chemically pure crystals of neutral sodic tartrate in 480 grams of liquor sodæ—specific gravity, 1.14.

To this add the copper solution, a little at a time; then dilute the mixture with distilled water to 1000 grams.—Brooks.]

This test is convenient in so far as addition of only one solution is necessary; this advantage, however, is offset by the instability of the solution. Furthermore, it also possesses the defects mentioned under test No. 1.

3. Moore's Test.—The urine is rendered strongly alkaline with potassium hydrate and boiled. The presence of sugar is manifested by a distinct caramel odor and a more or less intense brownish discoloration. The test is more delicate when the urine is *overlaid* with potassium hydrate and heated only at the point of contact. There then forms a distinct brownish-red ring. Although this test is not very delicate, it can be recommended for purposes of orientation. *Distinct reaction is not shown with amounts of sugar less than 0.5 per cent.*

4. Böttger's Test.—After strong alkalinization and addition of as much basic bismuth subnitrate as can be held upon the point of a penknife, the urine is boiled. In the presence of grape-sugar a deep-black precipitate of bismuth oxid occurs.

As regards the delicacy and reliability of this test, the remarks made under test No. 1 are applicable here.

5. Nylander's Test.—This is a most valuable modification of the former (No. 4). The solution is composed of 2.0 bismuth subnitrate, 4.0 Rochelle salts, and 100.0 of an 8-per-cent. solution of sodium hydrate. One part of this solution is added to 9 parts by volume of the urine, and the mixture boiled for a time. The reaction begins as a grayish-black coloration of the whole mixture, which soon becomes a deep black.

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[1] See also test No. 10.

The test is far more delicate than those already mentioned, and it reveals sugar in ordinary urines in amounts of 0.05 per cent., but in concentrated urines only in amounts of 0.1 per cent. upward.

A *faint* reaction may be produced even in nonsaccharin urines, especially when drugs, such as rhubarb and senna, antipyrin, salicylic acid, camphor, chloroform, chloral hydrate, saccharin, and turpentine have been ingested. All of these substances may reduce cupric and bismuth oxid to a certain degree.

With due consideration of these circumstances, Nylander's test can be *strongly recommended* for the use of the practitioner. It is easily performed and the solution keeps perfectly for many months.

6. Von Jaksch's Phenylhydrazin Test.—The urine is diluted with an equal volume of water, 2 penknife-pointfuls of phenylhydrazin hydrochlorate and 4 penknife-pointfuls of sodium acetate added, and boiled for twenty minutes in a water-bath. After cooling in water either a precipitate is at once produced which microscopically is composed of yellow needles or the crystals are found only in the sediment.

While the test is quite delicate, it is not always decisive for sugar, because, as has already been stated (page 312), glycuronic acid also gives with it similar crystalline formations which are distinguishable only by their lower melting-point. In practice only an abundant precipitate of yellow crystals can be considered conclusive, since a slight reaction occurs in almost every normal urine. *Therefore, in reliability and convenience, it is far inferior to the Nylander test.*

7. Hoppe-Seyler's Method.—The reagents employed are 0.5-per-cent. solution of nitrophenolpropionic acid in sodium hydrate. Ten drops of urine are boiled for one-fourth minute with 5 cubic centimeters of this reagent. The appearance of a *dark-blue* color shows reducing substances (not less than 0.5 per cent. of sugar). The presence of albumin does not interfere with the test. Urine free from sugar gives a greenish color only after the addition of 1 cubic centimeter; distinct blue color only on addition of much larger amounts, and even then not as with sugar urine.

8. The Fermentation Test *is unquestionably the most reliable method for the detection of sugar in the urine, and should be employed in every case in which any doubt exists.* It invariably shows glucose in amounts of 0.1 per cent. and upward, and can easily and conveniently be applied. Its sole disadvantage, however, is that, when small amounts of sugar are present, a decision is obtained only after from eighteen to twenty hours.

For performance of the test the so-called fermentation tube is employed. *Einhorn's apparatus* (Fig. 108) is the most prac-

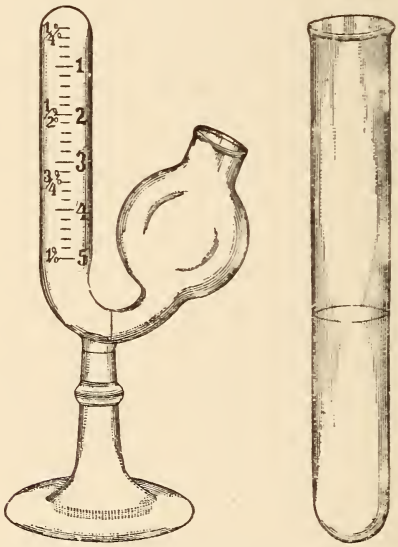


Fig. 108.—Einhorn's Fermentation Saccharometer.

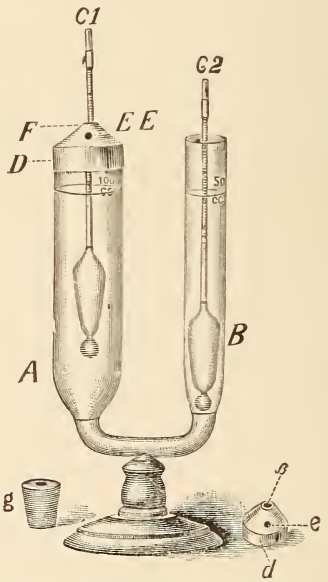
tical, because by its use a quantitative estimation can also be made with approximate accuracy. The bulb is filled with the urine which has been thoroughly mixed with a piece of fresh pressed yeast [one-sixteenth of a cake of Fleischmann's compressed yeast. The accompanying tube is filled up to the mark —, which indicates 10 cubic centimeters. The yeast is then added and thoroughly mixed with the urine with a glass rod, and the mixture thus obtained poured into the apparatus.—Brooks.] By careful tilting of the apparatus the mixture is allowed to flow into and *completely fill the upright arm,* which

can easily be done. No air should remain at the closed top of the tube. The apparatus is now set aside for from fifteen to twenty hours, at a temperature of 15° C. (60° F.), after which time the volume, if any, of carbonic acid gas formed and the percentage of sugar equivalent thereto can be read off from the scale etched upon the upright arm. [By placing the apparatus in an incubator, better results are obtained.—Brooks.]

As Einhorn's apparatus does not show more than 1 per cent. of sugar, urines containing more than this amount must be diluted. Given a urine with a specific gravity of 1.018 to 1.022, it is advisable to dilute two times; one with a specific gravity of 1.022 to 1.028, five times; and, above this, ten times. The percentage indicated by the scale must then be multiplied by 2, 5, or 10, according to dilution, which will give the percentage of sugar present in the undiluted urine.

[**Stern's Urinoglucosometer.** — This apparatus (Fig. 109) consists of one small *(B)* and one large glass tube *(A)* united at their bases to form a U. The large tube is gauged to 100 cubic centimeters and the small tube to 50 cubic centimeters. Both tubes are provided with perforated metal caps *(EE, e)*. Fifty cubic centimeters of urine are placed in the smaller tube and the latter tightly closed with the rubber stopper *(g)*. Place 3 or 4 grams of compressed yeast into the larger tube and then add urine to the mark *100 c.c.* Insert the urinometers, cover the tubes with the metal caps, and set the apparatus aside at a temperature of 80° F. for from twelve to fifteen hours. After this time remove the metal cap from the larger tube and stir the urine to liberate the CO_2. Replace the cap and allow the cloud to subside. When the flocculi have settled the specific gravity of the urine in both tubes is determined by reading the scale on the urinometers from the flat top of the metal caps. As each degree of specific gravity lost equals 0.2196 gram of glucose per 100 cubic centimeters of urine, the difference in the specific gravity of the contents of both tubes multiplied by 0.2196 equals the percentage of sugar present. For example, if the specific gravity of the urine in the small tube (unfermented) is 1.036 and that in the large tube (fermented) is 1.020, the difference is 16. $16 \times 0.2196 = 3.1536$ grams per 100 cubic centimeters, or 3.1536 per cent.—Brooks.]

The **method of Moritz** is very practical. An ordinary test-tube is filled about three-fourths with a mixture of yeast and urine and then made to overflow by addition of mercury. It is then closed with a perforated rubber cork through which a U-shaped glass tube is passed. The urine fills the bent tube. The air-free apparatus is then inverted, so that the mercury occupies the lower part of the test-tube and U-tube. The mercury is forced out by the carbonic acid gas which accumulated at the



[Fig. 109.—Stern's Urinoglucosometer.]

closed end of the tube and thus offers positive evidence of the presence of sugar. The addition of 2 per cent. of yeast suffices for the test.

In the fermentation test "spontaneous fermentation of the yeast" may be mistaken for *minute* quantities of sugar. In practice, however, this point can almost always be ignored. If, however, it is desirable to be absolutely certain, a second tube should be filled with a mixture of yeast and perfectly normal urine for control. If this tube presents no trace of fermentation, then the CO_2 developed in the other tube can be accepted as absolute evi-

dence of the presence of sugar. This deceptive development of gas can also be almost entirely avoided by previously boiling the urine (before addition of the yeast) for ten minutes.

A criticism of this method will be found under tests Nos. 10 and 11 (pages 323 and 325).

9. Detection with the Polariscope.—Next to the fermentation test, the polariscope is of chief importance in determining

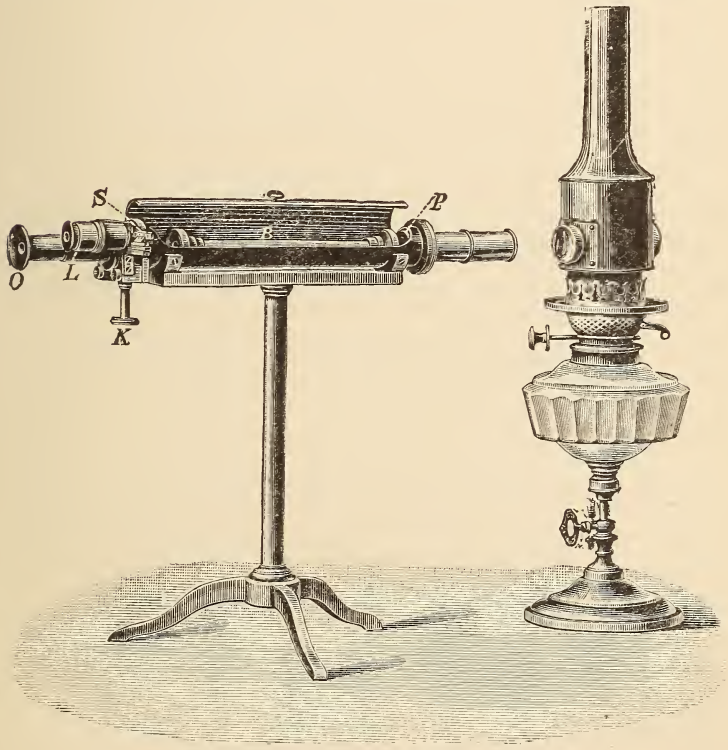


Fig. 110.—Polarization Apparatus.

the presence of glucose in the urine. The so-called "half-shadow" apparatus is usually employed, by means of which the specific "dextrorotatory" action of glucose can readily be recognized; 0.1 per cent. can be positively determined.

Polarization possesses the advantage over all other previously mentioned methods in that it also permits quantitative estima-

tion. It should be remembered, however, that the urine must be *perfectly clear,* since any cloudiness absorbs light. Clarification is most easily secured by addition of one-tenth volume of lead acetate, which must be considered in the final calculation. Furthermore, albumin, which is sometimes present, must be removed by the method above mentioned.

For the performance of this method, the half-shadow new apparatus (Fig. 110) manufactured by Schmidt and Haensch, of Berlin, is preferable. This apparatus, unlike that of Mitscherlich, does not require sodium light, but can be employed with the ordinary white light of a gas or petroleum flame. The apparatus shows glucose (and albumin) in amounts of 0.1 per cent.

Procedure.—The lamp should be placed about thirty centimeters from the apparatus, in such a manner that the best light enters the illuminating system of the apparatus. When the observer looks through

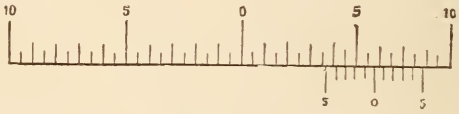


Fig. 111.

the instrument, before the tube *B* is placed in position, a clear, circular field, divided into two equal parts by a distinct central perpendicular line, must be seen. If the field is hazy, the telescope *(O)* should be drawn out or pushed in until this division line of the shadow-half-nickel *(P)* is perfectly defined. Reading of the scale *(S)* is done by means of the lens *(L),* which is drawn out or inserted until the scale can be distinctly recognized. If the zero point of the vernier is exactly upon the zero point of the scale *(S)* in such a manner that both lines form a straight line, then the apparatus is exactly in the *O* position and both halves of the field are equally illuminated. If the pinion *(K)* is turned slightly from left to right, the left half of the field becomes dark, the right illuminated; if, on the other hand, the pinion is turned from the *O* point slightly from right to left, the right half becomes dark and the left illuminated.

If the observation tube *(B)* is now filled with sugar urine and placed in the apparatus adjusted to the *O* point, the field no longer appears clear; it is, therefore, absolutely necessary first to restore the former sharpness by lengthening or shortening the telescope. One-half of the field will then be dark and the other bright. *In order to determine the amount of sugar, it is necessary to turn the screw (K) until both*

halves of the field appear exactly the same. A slight turn to the left or right must then produce the same differences in the field as when the apparatus was in the *O* position, minus tube and fluid.

The scale then gives directly the percentage of urine sugar. The reading (see Fig. 111) is done as follows:—

Each interval of the scale equals 0.5 per cent. of urine sugar; above the vernier there are four such intervals divided into five parts. Assumed that the position of the scale with the vernier gave the register shown in Fig. 111, on page 322, with uniformity of both halves of the field, it will at once be seen that five degrees (equals 5 per cent.) have passed the *O* point of the vernier; furthermore, an additional interval (equal to 0.5 per cent.) has passed the *O* point of the vernier, which now rests between the eleventh and twelfth interval. The *O* point has not quite reached the latter; the decimal percentage is read by observing which line of the vernier (to the right of the *O* point) *forms a continuous line with a line on the scale.* In our example, the third line of the vernier is continuous with one line of the scale; consequently, 0.3 per cent. must be added to the 5.5 per cent. already obtained, giving a total of 5.8 per cent. of urine sugar. This result has reference to the employment of an observation tube 200 millimeters in length; if a 100-millimeter tube is used, the result must be multiplied by 2, and, with use of a small tube of 50 millimeters, by 4.

If the urine contains albumin (which rotates to the left), a second polarization must be made after elimination of this substance by boiling and filtration of the urine. The difference between the first and second polarization gives the percentage of albumin, while the second polarization gives the correct percentage of urine sugar contained in the fluid. If, for example, the first polarization gave 3.7 per cent. and the second 3.9, the total result would be 3.9 per cent. of urine sugar and 0.2 per cent. of albumin.

10. Fehling's Method for the **quantitative** estimation of sugar is based upon the fact that exactly 5 milligrams of glucose reduce one cubic centimeter of Fehling's solution.

This solution is best preserved by keeping both components separately and mixing them fresh when required for use. For preparation of *Solution I,* dissolve 34.639 grams of noneffloresced crystals of pure copper sulphate, crushed between filter-paper, in from 200 to 300 cubic centimeters of water by aid of gentle heat, and dilute the solution at ordinary temperature to 500 cubic centimeters. The vessel should be carefully closed with a ground-glass stopper.

Solution II contains 173 grams of crystallized Rochelle salts, in 350 cubic centimeters of pure sodium hydrate,—specific gravity, 1.14 (or 50 grams of caustic soda),—diluted to a total volume of 500 cubic centimeters. The vessel should be closed with stopper coated with paraffin.

After a sample of Fehling's solution has been tested by boiling, to see that no precipitate occurs, while such at once occurs on addition of sugar urine, the test can best be employed as follows:—

The sample of urine to be examined is diluted with from 4 to 10 volumes of water, according as the specific gravity is 1.032 or over, and placed in a burette. Five cubic centimeters of Solutions I and II are heated to boiling in a porcelain dish and urine added, $^1/_{10}$ cubic centimeter at a time, under constant stirring. The titration is continued as long as the faintest blue coloration can be noted in the dish.

If we assume that 15 cubic centimeters of a fourfold diluted urine were used, the estimation is at once very simple. We know that 1 cubic centimeter of Fehling's solution is reduced by 0.005 of sugar. In our example 15 cubic centimeters of urine have reduced 10 cubic centimeters of Fehling's solution. Accordingly:—

$$15.0 : 0.05 = 100 : x \text{ or } x = \frac{5}{15} = 0.33$$

Since the urine was diluted with four volumes of water, we obtain: $4 \times 0.33 = 1.32$ per cent. of sugar.

[Percentage of Sugar in Urine as Indicated by the Quantity of Urine Required to Decolor Exactly One Cubic Centimeter of Fehling's Solution. (After Wormley.)

Undiluted Urine.		Diluted Urine 1 in 10.	
C. C. Urine.	Grape-sugar Percentage.	C. C. Urine.	Grape-sugar Percentage.
0.1	5.0	0.4	12.5
0.2	2.5	0.5	10.0
0.3	1.66	0.6	8.33
0.4	1.25	0.7	7.14
0 5	1.00	0.8	6.25
0.6	0.83	0.9	5.55
0.7	0.71	1.0	5.0
0.8	0.62	1.4	3.5
0.9	0.55	1.8	2.7
1.0	0.5	2.0	2.5
		2.5	2.0
		3.0	1.6
		4.0	1.25
		5.0	1.00]

The necessary dilution of the urine can usually be determined by the specific gravity, since the sugar content is generally the greater the denser the urine. In a specific gravity of 1.030 it is advisable to dilute five times, and in greater density ten times. In order to secure the greatest possible accuracy, it is recommended to repeat the titration once or twice. The test gives decidedly more accurate results than the method of determination with Einhorn's fermentation tubes, because the influence of the CuO present in normal (especially concentrated) urines is very much reduced by dilution of the urine.

If the diabetic urine contains more than 0.2 per mille of albumin, it is necessary to remove this before estimating the sugar, since, the more the albumin content approaches the above percentage, the more slowly the oxydul is precipitated from the fluid.

11. J. Schütz's Araëo-saccharometer is a method deserving of commendation in general practice. The method is based upon the fact that a vessel, filled with diabetic urine and immersed in water, floats at different levels *before* and *after* fermentation of the sugar. An araëometeroid vessel with a long neck can be empirically graduated, by means of which a quite accurate estimation of the percentage of sugar in diabetes can be made.

To perform the test fill the vessel with urine to the full mark and add 1 gram of compressed yeast and enough shot to cause the spindle to sink in the water to the 0-per-cent. sugar mark [see Stern's apparatus; it is simple and very convenient]. Then by careful shaking thoroughly mix the yeast with the urine and set the tube containing the mixture aside to ferment for from twenty-four to thirty-six hours at room temperature. At the end of this time reimmerse the spindle in water and read off the specific gravity and the percentage of sugar.

Many control tests have convinced the author that this method possesses many advantages, although, as regards accuracy, he has not found it as free of objection as Schütz states. However, this method is certainly preferable to Einhorn's method of estimation.

[**Lohnstein** has recently devised a **fermentation saccharometer**[1] which indicates *directly* the amount of sugar in the urine from 0 to 10 per cent. *without dilution* of the specimen. Accord-

[1] [Deutsche medicinische Wochenschrift, 1900, No. 31.]

ing to the author, it works more quickly than any other fermentation instrument, and in accuracy it equals even the most expensive polarization apparatus. Dr. A. Spaethe, of Berlin, working in Senator's polyclinic, has compared the results obtained with this instrument with those secured with Einhorn's, Fiebig's, and the older Lohnstein apparatus. As control apparatus he employed the half-shadow polarization instrument of the polyclinic. He concludes that the Lohnstein instrument unquestionably deserves the preference, and states: "With this apparatus we obtain most satisfactory and practical results in every particular, and we can warmly recommend it to the practitioner particularly because of its *simplicity* and *practicability*." J. Meyer, P. Meissner, C. S. Engel, O. Loevinson, F. Goldmann, Dr. Weil, and P. Stroomann also speak highly of it.—Brooks.]

Blood Tests.

The *blood* of diabetics gives two reactions which may be briefly mentioned here because they may occasionally be of diagnostic significance.

1. Bremer's Test.—Dried preparations of diabetic blood fixed at 130° C. are stained much more intensely after three minutes' contact with 1-per-cent. solution of methylene blue than blood preparations obtained from healthy individuals. Normal blood, also, may manifest the reaction if treated with diabetic urine. The reaction is not wholly free of objection, because it may not appear in many cases of diabetes or pronounced glycosuria. Bremer himself noted the absence of the reaction in a patient who excreted urine containing 6 $^1/_2$ per cent. of sugar. On the other hand (and the same is true of the following tests), it may be of value in many cases of coma in which an examination of the urine is impossible. An explanation of this phenomenon is still lacking. It is very probable that the reaction is connected with the abnormal acid property of the urine (Schneider).

2. Williamson's Test.—Twenty cubic millimeters of freshly drawn blood are taken in a very slender test-tube and 40 cubic millimeters of 6-per-cent. caustic potash and 1 centimeter of watery solution of methylene blue (1 to 6000) added. A control tube containing normal blood is treated in the same manner.

If the tubes are now heated in a water-bath, the diabetic blood solution will become decolored (colorless or pale yellow), often within from one to two minutes, at the latest after five minutes, while a similar decoloration in the control tube occurs only after continuous heating for from ten to twenty minutes.

Finally, in the examination of *diabetic urine* there are two more important reactions which should always be conducted, because they are of valuable prognostic significance. They are **Gerhardt's iron chlorid reaction** and the **acetone test.** The former is of importance in many acute infectious diseases, as well as in severe forms of diabetes.

1. The Iron Chlorid Reaction of Gerhardt.

Procedure.—Iron chlorid is added to fresh urine, 1 or 2 drops at a time, until a Bordeaux-red color appears, which is due to the presence of *aceto-acetic* (diacetic) *acid.* At first a chocolate-colored precipitate of iron phosphate is formed. On addition of sulphuric acid the color at once disappears. After acidulation of the urine with sulphuric acid, the diacetic acid can be extracted with ether, and this tested for the iron chlorid reaction.

The significance of Gerhardt's test depends upon the fact that its intense response is a *signum mali ominis,* which not infrequently points to impending diabetic coma. According to the researches of Sadelmann and Minkowski, there can be no doubt that this is produced by an acid intoxication with oxybutyric acid. If the iron chlorid reaction is strongly marked, it may safely be assumed that β-oxybutyric acid is present.

2. Legal's Acetone Test.

A few drops of fresh solution of sodium nitroprussiate are added to the urine and saturated sodium hydrate until a distinct alkaline reaction is produced. After the purple color produced by this addition has been succeeded by a pale yellow, carefully add a few drops of saturated acetic acid. If a bright-purple or carmin color appears, the presence of acetone is proved.

If it is abundantly present, the urine not infrequently has a strong odor of apples. Aside from diabetes mellitus, it occurs in high fever, gastric and intestinal cancer, acute infectious diseases, and febrile gastric disturbances of children.

The Diazo-reaction and Burgundy Reaction.

For the sake of completeness, we may again briefly mention the **diazo-reaction** (Ehrlich's) and the **Burgundy reaction** (Rosenbach's).

Ehrlich's reagent consists of: 1. Sulphanilic acid, 5.0; hydrochloric acid, 50.0; and distilled water, 1000.0. 2. Sodium nitrate, 0.5 in distilled water, 100.0. In performing the test 250.0 cubic centimeters of solution No. 1 are mixed with 6 cubic centimeters of solution No. 2. One part of this mixed reagent is placed in a test-tube with 1 part of urine and about one-eighth the volume of ammonia added. On shaking the mixture there occurs in many febrile diseases a red coloration of variable intensity. This reaction is observed especially in typhoid fever, severe pulmonary tuberculosis, and pneumonia. Disappearance of the reaction is said to be indicative of a favorable prognosis. [Ehrlich's diazo-reaction enables us to foretell certain complications. It is sometimes manifest as early as the third or fourth day, but its appearance may be delayed until the beginning of the third week. If it ceases suddenly in typhoid unattended by concomitant amelioration, some secondary infection or renal lesion must be suspected. On the other hand, an increase in intensity indicates an aggravation of the disease. Its reappearance during convalescence is indicative of a relapse. The diazo-reaction is also observed in typhus fever, acute tuberculosis, pyemia, septicemia, scarlatina, measles, and occasionally in pneumonia. In these diseases, except tuberculosis, it does not seem to be of any especial prognostic significance. In a case of scarlatina recently observed by the writer this reaction was very marked during a number of days. The case subsequently made a good recovery.—Brooks.]

Rosenbach's Reaction is manifested by the appearance of a deep Burgundy red produced in the usually previously reddish urine, on continued boiling and addition of nitric acid drop by drop. The reaction is usually manifested in severe intestinal disturbances associated with indicanuria.

MICROSCOPIC EXAMINATION OF THE URINE.

Microscopic examination of the urine is directed chiefly to the sediment, which is designated as *organized* or *unorganized* according as it is composed of cells or their derivatives or of crystalline and amorphous chemic compounds.

The sediment of the urine is obtained either spontaneously by allowing it to stand in a conic glass or it is precipitated quickly by the centrifuge. The first method is the usual one and suffices for general practice. In order to secure the sediment as

rapidly as possible, it is advisable to decant the top of the urine collected in a large urine glass and to pour only the *lower* portion, which on standing is richer in formed elements, into a conic glass after the urine has been shaken and allow the sediment to settle. The sediment will form quickly or slowly and be dense or nebulous in character according to the amount of suspended elements present. If the urine is very cloudy and rich in formed elements, it will be easy in every instance to secure with a pipette sufficient sediment for examination; if only a slight deposit is present, much care is necessary. Under such circumstances, *the pipette, held between the thumb and middle finger and closed tightly at the upper end with the tip of the index finger, should be passed to the bottom of the conic glass, care being taken to avoid any dispersion of the sediment.* The tip of the index finger is then raised slightly, so that a little of the sediment is sucked up into the pipette, and at once replaced; the pipette is carefully withdrawn and wiped off—*while still closed with the finger-tip—with a cloth to remove all adhering fluid.* This is necessary in order to avoid dilution of the sediment. A small drop is now allowed to run from the pipette upon a glass slide and covered with a cover-glass. The latter should not float upon the fluid, because the microscopic field is thereby disturbed and rendered indistinct by the constant trembling movements.[1]

[Better results will be obtained by the following method: According to the density of the sediment,[2] by means of a gently tapering pipette transfer 5 or 10 drops to a large clean slide and spread it out into a thin, uniform layer for examination. Now place the slide upon the stage of the microscope—which should, of course, be perfectly level—and examine the sediment *without* a cover-glass.

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[1] For the preservation of urine sediment, a 1-per-cent. solution of osmic acid can be used. Two or 3 drops of the sediment are added to 3 cubic centimeters of this solution. After one or two days, when the sediment has completely settled, the acid is drawn off and replaced by pure glycerin. Urine sediment prepared in this manner keeps for a long time unaltered. The morphologic elements, after removal of the osmic acid and washing, can be stained (Kuttner).

[2] [Even when no sediment is visible to the naked eye microscopic examination of some of the urine from the bottom of the vessel containing it will show the presence of organized and unorganized elements.—Brooks.]

In a search for *casts* the method of examining the urine sediment under a cover-glass and with high-power lens often gives negative results even when quite a number of casts are present in the specimen under examination. Consequently, *urine sediment should always be examined first with low-power lens* ($^2/_3$) and high eyepiece (1 inch), the substage lowered, or—in the absence of this attachment—the mirror tilted so as to *avoid too brilliant illumination.* Use plane (not concave!) mirror. If these precautions are not observed, the delicate hyaline and finely granular casts and so-called "cylindroids" (which have the same significance as casts), will often be overlooked, and the microscopic examination in this respect pronounced negative.

Usually, a moderate amount of practice will enable one to determine the character of the casts with the $^2/_3$ objective and 1-inch eyepiece. Should there be any doubt, however, as to the exact nature of the casts seen,—whether hyaline, granular, blood, pus, epitheliated, epithelial, fatty, etc.,—place the casts precisely in the center of the field of the low-power lens, raise the substage to the level of the under surface of the slide, change to the high-power ($^1/_5$ or $^1/_6$) lens and *concave* mirror and gently lower the lens, by means of the coarse adjustment, until the object comes into view. If the lenses are well centered, this can easily be done. As soon as the *shadow of the object* comes into view, change to the fine adjustment. By gentle movement of the fine adjustment details can now be studied. With a little care this can be done without immersing the lens in the fluid, provided lenses not higher than $^1/_5$ are used.

The satisfactory examination of renal elements, blood, and pus-corpuscles, etc., when present in small number, requires the high-power ($^1/_5$ or $^1/_6$) lens, concave mirror, and full illumination. Here, also, a cover-glass is not necessary, though there is no objection to its use if the sediment is abundant or has been concentrated by appropriate methods of sedimentation (centrifuge). If lenses higher than $^1/_5$ inch are used, a large cover-glass will avoid much embarrassment from immersion of the lens in the fluid. When present in large amount pus and blood can readily be made out with low ($^2/_3$) power. Examination for

renal elements should always be conducted with high powers—at least $^1/_5$-inch objective.

Should the organized elements of the urine sediment be obscured by the presence of phosphates or alkaline urates, a few drops of acid will clear them away; if amorphous urates are the disturbing element, gently heating the slide of urine over a Bunsen or alcohol flame will dissipate them. If the urine cools and a precipitate of the amorphous urates re-forms on the slide before the examination of this particular portion of the sediment is completed, repeat the heating and continue the examination. A few drops of caustic alkali solution will also clear up the urates.—Brooks.]

[**Urinary sediments** containing organized elements may conveniently be **preserved** by carefully decanting the urine from the sediment and filling the vessel with a watery solution of acetate of potash having a specific gravity of from 1.050 to 1.060, made by adding acetate of potash to distilled water until the specific gravity named is reached. To this is added 0.5 per cent. of deliquesced (by heat) crystals of carbolic acid. After the sediment has again subsided to the bottom, decant a second time and add fresh acetate-carbolic solution. If necessary, this may be done a third time. By this method the sediment can be preserved fairly well for months.—Brooks.]

ORGANIZED URINARY SEDIMENTS.

Before discussing this subject in detail it appears to be desirable briefly to sketch the histologic relations of the kidneys and urinary tract, because a conclusion as to the participation of the various portions of the urinary apparatus can be drawn from the morphologic elements present in the urine only when the histologic structure is constantly borne in mind.

The kidneys are tubular glands composed of innumerable ducts—the "renal canaliculi." The kidney is divided into a cortic and a medullary substance by the tortuous course of the peripheral and the straight course of the central tubuli. Each tubule begins at the bulbus glomerulus: *i.e.*, the vascular tuft surrounded by Bowman's capsule. After a slight constriction the tortuous [so-called proximal convoluted portion—Brooks] tubule begins, with the walls of which the external layer of Bowman's capsule is continuous. The convoluted tubule then passes on to form the descending arm of Henle's loop, which in turn passes on to form the ascending arm, which is united with the *collecting tubule* by the intercalary [so-called distal convoluted] portion.

During this course of the urinary tubule its *epithelial* lining undergoes many changes. In the convoluted portions the epithelia are usually low, thick, and conic shaped, with granular protoplasm; in the descending arm of Henle's loop, clear and flattened; in the ascending arm, like those of the convoluted portion, but usually not so high; in the intercalary portion and the collecting tubule, usually cylindric. The nucleus of the cell is distinctly oval and shows a nucleolus. *The epithelia of the tubules are distinguished* from the *superficial epithelia of the true* urinary passages (renal calices, pelvis, and ureters) *by their more polyhedral form and smaller size.* The epithelium of the renal calices, like that of the bladder, forms a layer composed of flattened cylindric cells. Many transitional forms occur, however. It must be especially emphasized that *the epithelia of the conducting urinary passage and of the bladder present exactly similar appearances.* Since epithelia from the urethra and vagina are also mixed with the urine, it must further be mentioned that the

Fig. 112.—Epithelium from the Urinary Tract (Obtained by Scraping the Mucosa). × 350.

a, Renal pelvis. *b*, Ureter. *c*, Bladder. *d*, Excretory duct of prostate.

epithelium of the male urethra in the pars prostatica is like that of the bladder. Further on, however, it is distinctly cylindric, and only after the fossa navicularis is reached does it become decidedly flattened [squamous]. The female urethra may contain squamous or cylindric epithelia (Stöhr).

The glandular cells of the prostate are of a low cylindric type, while the excretory ducts of the prostate are provided with transitional epithelia. The epithelia of the ejaculatory duct and that of the tubules of Cowper's gland are cylindric.

The vagina is covered with lamellated squamous epithelium.

Of organized constituents, the following occur:—

1. Red Blood-corpuscles.—These appear in the urine after every hemorrhage occurring upon the mucous membrane of the urinary apparatus. In fresh acid urine they preserve their nor-

mal size, form, and color. Various alterations, which are due partly to imbibition, partly to shrinkage and extraction of the hemoglobin, begin only after some time, as the result of the action of urinary salts and water. The cells then often appear enlarged or small and crenated, or, finally, as delicate, easily overlooked, pale rings (shadows [or ghosts]). *Rouleaux formation is never observed.* Not infrequently, however, they adhere to urinary casts, or they form casts without demonstrable cement substance between the densely massed cells (see Fig. 113, *bl*).

Fig. 113.—Acute Hemorrhagic Nephritis. × 350.

Small and large squamous epithelium, hyaline casts (at the margin). *g*, Finely granular cast. *bl*, Red blood-corpuscle cast. *e*, Tubular epithelium (arranged in cast form). Here and there are blood-corpuscle rings ("shadows" [ghosts]).

The microscopic detection of red blood-corpuscles decides the often open question whether a hematuria or hemoglobinuria exists. If unaltered red blood-corpuscles are found in the sometimes pale red, sometimes dark-reddish-brown urine, then hematuria exists; if such are absent from a urine in which blood-coloring matter has unquestionably been determined by other methods, then hemoglobinuria is present.

As to the *location of the hemorrhage,* aside from important clinical symptoms, other morphologic elements must decide. The coincident presence of casts and renal epithelia speaks in

favor of *renal hemorrhage.* In *vesical hemorrhage* the above-mentioned elements are not found, but numerous squamous epithelia are present. Numerous fragmented red blood-corpuscles, which, perhaps, are deserving of diagnostic attention, are occasionally observed in renal hemorrhage. In addition, the above-named macroscopic differences are to be considered.

[Guide to the Origin of Blood in Hematuria. (After Oertel.)

Origin.	Quantity.	Differential Points.	May Occur in:
Kidney.	Usually comparatively small.	Clots usually absent. Associated with blood-casts, epithelial and hyaline casts, renal epithelium. Intimately mixed with urine. Many swollen (loss of hemoglobin) phantom corpuscles. Sediment slight [or abundant].	Acute and chronic nephritis. Malignant growths. Renal calculus, tuberculosis, embolism, abscess, acute febrile processes, hemophilia, and in filariasis [malaria] and distomiasis [?]. Frequently in poisoning from turpentine, carbolic acid, etc.
Pelvis of kidney and ureters.	Variable.	Absence of casts of any kind or renal epithelium. Fibrinous molds of ureters may be present. Pus-cells in calculus.	Disease of pelvis, calculus, etc.
Bladder.	Frequently large.	Blood-cells well preserved—unless urine is ammoniacal. Clots frequent. Heavy sediment [often scanty]. Pus in cystitis. In papilloma and malignant growths may be shreds of such tissue. If from neck of bladder, appears at end of micturition.	Stone, cystitis, tumors, varicose veins of vesical neck [distomum hæmatobium], etc.
Urethra.	Small.	May be expressed. First part of micturition.	Urethritis, trauma, etc.

—Brooks.]

2. Leucocytes.—These are found normally in small numbers in almost every urine. Their frequent occurrence is pathologic, but they are often observed in the most varied disturbances. In addition to many pathologic conditions of the external and

internal genitals and in all catarrhal states of the bladder and ureters, they are usually present also in genuine renal diseases. The cells and nuclei present the ordinary size and form, and usually show in dry preparations neutrophilic granulation. Very frequently they are seen adhering to renal casts. In just these instances they may readily be mistaken for epithelia of the renal tubules. In addition to the marks of distinction immediately to be mentioned, it should be noted especially that the leucocytes are round and usually *characterized by a polymorphous* nucleus. Staining of the leucocytes with Ehrlich's staining solutions shows no uniformity in the character of the cells. Sometimes numerous small mononuclear cells are seen, which, perhaps, appear with the lymph [lymphocytes] and have entered the urinary tubules as a result of existing epithelial defects.

3. Epithelia.—Isolated *squamous epithelia* are not infrequently found in normal urine, especially in women. Numerous epithelia of this type always indicate some pathologic process. They are found in all acute and chronic catarrhal states of the urethra and bladder. They are large, often polygonal or rounded at the corners, usually flattened, occasionally somewhat swollen cells with a large and generally sharply defined, faintly granular nucleus (Figs. 113, 114, and 116).

Club-shaped monocaudate or polycaudate nucleated epithelia were formerly often very wrongly interpreted as characteristic *renal pelvic* epithelia. Exactly the same caudate forms may come from the ureters as well as from the bladder itself. Swollen cylindric epithelial cells with one or two prolongations may also be derived from the excretory ducts of the prostate, and are not rarely found in the mucous shreds so frequently discharged from this locality.

The determination of epithelia from the *renal tubules* is much more certain. The inexperienced observer frequently confuses them with colorless blood-corpuscles [leucocytes, pus-cells —Brooks], from which they *sometimes* cannot be distinguished. As a rule, however, they are so distinctly characterized by their *polygonal form* and single large, round or somewhat oval nucleus that they can be diagnosticated with certainty. Their occurrence is of great semeiotic significance, for according to the quantity of the excreted elements they always certainly

indicate a more or less intense epithelial desquamation. Finally, the renal epithelia occur singly or in small and large groups in severe (especially acute) nephritis in the form of "epithelial tubes." These are cylindric formations composed of densely packed epithelia superimposed like shingles or arranged in mosaic form without distinct cement substance (Fig. 113, *e*, and Fig. 114, *e*, page 332).

The epithelia are often intact, but they are not infrequently "albumin clouded," or transformed into the genuine, more or less large **fat-granule cells** resembling the colostrum-corpuscles of

Fig. 114.—Severe Acute (at first decidedly hemorrhagic) Nephritis, which Ended Fatally in Four Weeks. × 350.

h, Hyaline cast. *g*, Granular cast. *w*, Waxy cast. *e*, Epithelial cast. *ep*, Free renal epithelia. Also two finely granular, uniformly fatty renal epithelia.

milk. These often present, in addition to numerous minute fat-globules, a quite distinct nucleus; not infrequently, however, the cell is so densely filled with small and large fat-droplets that the nucleus is wholly obscured. Such a cell not rarely obtains a peculiar dark appearance as a result of the many intensely refractive fat-globules heaped upon each other (Fig. 115, *k*).

The cloudiness is designated as albuminous when the individual granules are only slightly refractive and soluble in dilute potassium hydrate and acetic acid and *insoluble* in ether. On the other hand, the

granulations of the granule cells, which are produced by genuine fatty degeneration of the proteid substances, are characterized by their insolubility in potassium hydrate and acetic acid and their solubility in ether and alcohol, blackening in osmic acid and brilliant-red coloration by sudan III.

The granule cells are found particularly numerous, both free and adhering to casts, in the large white kidney. They are less often observed in other forms of nephritis, and then usually in *severe* acute inflammation. In this instance their abundant presence is of decidedly unfavorable prognostic significance.

Fig. 115.—"Large White Kidney." × 350.

h, Hyaline cast. *g*, Spiral cast. *w*, Waxy cast. *f*, Fat-granule cast with: *n*, fat needles. Still finer needles of this type upon the neighboring fat-granule spherule. *k*, Fat-granule cell. *l*, Leucocyte. *s*, Vaginal epithelium. *t*, Fat-droplets.

4. Renal Casts.—By these are understood delicate, cylindric formations of variable length, thickness, and external appearances. They were found first by Henle (1844) in the urine and in the kidney and described as important accompaniments of renal affections. In addition to the epithelia of the kidney tubules, they occupy a prominent position among the organized sediments in the diagnosis of renal affections.

Three varieties are usually distinguished: **hyaline, granular,** and **waxy casts.**

The **hyaline casts** occur in very variable length (up to from 1 to 2 millimeters) and breadth (from 10 to 50 μ). They are delicate, translucent or transparent, glassy, perfectly homogeneous formations, and usually have a straight or occasionally a slightly tortuous contour, with parallel outlines. They are easily overlooked, but may be rendered distinct by various staining substances, such as iodin, carmin, picric acid, and basic anilin dyes added to the sediment in dilute solutions (see Figs. 113, 114, 115, and 116).

[Fig. 116.—Narrow Hyaline Casts. × 350. (After *Peyer.*)]

In icterus they present a yellowish-green color. Many kinds of morphologic elements, in addition to urates (especially urate of soda) and minute albuminous granules, are frequently found adhering to them, which, owing to their high semeiotic value, were justly designated by Frerichs as "heralds of the processes in the kidneys." Sometimes only isolated cells are found adhering to these casts, and not infrequently the latter are densely studded with them. Such forms constitute transitions to granular casts. Hyaline casts are easier to recognize by adherence of cells, and also in consequence of the evidence of fatty changes which are not infrequently to be noted in them.

Granular casts also occur in very variable size. Their surface is sometimes finely granular, especially when they are composed of densely arranged sodium urate or fine albuminous granules; sometimes coarsely granular, when they consist of red and colorless blood-cells or epithelia from the renal tubules. Under the latter circumstances it is usual to designate them as *red blood-corpuscle* and *epithelial casts (epithelial tubes)* (see Fig. 113, *bl, e*; Fig. 114, *g, h, e*; Fig. 115, *f*; Fig. 117, *h, e*).

In many cases a clear view of the formation of such casts can be obtained. Not infrequently a smaller or larger portion is composed of

Fig. 117.—Chronic Bright's Disease (Chronic Parenchymatous and Interstitial Nephritis). × 350.

h, g, e, w, Hyaline, granular, epithelial, and waxy casts. *ep*, Renal epithelium. *vep*, Quite uniformly fatty renal epithelium.

densely arranged blood-corpuscles or epithelia, while the remaining portion appears purely hyaline. At other times no trace of a cement substance can be made out in the casts. While in the former instance one is constrained to assume that the matrix of the casts consists of hyaline material which is only in part densely studded with cells, in the latter instance one is tempted to conclude that the whole mass of the cast consists of cells without any cement substance. One will not go astray, however, if he assumes that a cement substance usually exists.

As the epithelia not infrequently undergo a metamorphosis into granule-cells, one or more exquisite fat-granule cells are occasionally seen adhering to the casts. In rare instances even

the whole surface of a cast is composed of densely arranged granule-cells, or by their coalescence and further fatty transformation the cast is densely covered with small and large fat-globules, the development of which from individual fatty metamorphosed epithelia is rendered probable or certain. Now and then more or less long fat-crystal needles appear in such fat-granule spheres and casts (Fig. 115, *f*, *n*).

Waxy casts are much rarer, and are usually observed only in chronic forms of nephritis. They also occur, however, in severe

[Fig. 118.—Epithelial Casts. × 350. (After *Peyer.*)]

and usually fatal acute nephritis [also after prolonged ether anesthesia, nephropexies, or manipulation of the kidney in cases in which apparently mild renal affections pre-existed—Brooks]. They are often very long and usually much broader than the first-mentioned forms. They are distinguished from hyaline casts by their extremely sharp, intensely refractive contour and *translucent* character. *As a rule, they are very resistant to acids, while the hyaline casts disappear on their employment.* Lugol's solution stains them sometimes reddish brown, and *subsequent* addition of sulphuric acid a dirty violet (Figs. 114 and 115, *w*).

The great semeiotic significance of renal casts is shown by the fact that, with few exceptions, they *always* point to the existence of inflammatory processes in the kidneys. As *exceptions* are to be noted the almost constant occurrence of delicate hyaline cylinders in catarrhal icterus, in which they are slightly bile-stained, as well as in many forms of albuminuria. For example, in addition to delicate hyaline, usually also a few finely granular urate casts are met with in the urine of fever and passive congestion, in severe anemia, leukemia, diabetes, etc.

In the majority of cases they are an indication of genuine nephritis, the exact nature of which is revealed by the accompanying cellular elements. The number of casts is usually in proportion to the degree of albuminuria, and the severity of the affection. However, numerous exceptions occur here. Not infrequently the casts appear at the *beginning of the nephritis before albuminuria is demonstrable;* and in healing of an acute *morbus Brightii* they *very commonly* persist after the albumin excretion has ceased. Shortly before death numerous very thick and long casts often appear. The number of casts is not infrequently increased also during a uremic attack.

A certain diagnostic interest is attached to the *casts* occurring in *diabetic coma.* They not infrequently appear even *shortly before* the attack, and constantly and often in large numbers during the coma in the form of short stumps of hyaline and dull, glistening granular forms. Külz first described their occurrence. Like him, the author *never* missed these characteristic casts in coma. If the attack subsides (which, as is known, occurs in very few instances), the casts may rapidly and completely disappear. It is worthy of mention that even when large numbers of casts occur the albumin tests may show only a slight clouding of the urine. [A similar occurrence is frequently noted after prolonged ether anesthesia.]

Aside from the very thick casts occurring in cases of weakness of the force of the heart and scanty urine, the seat of formation of which may be assumed as probable in the collecting tubules, it is *not permissible* to form further conclusions as to their local origin from the form of the casts. Even quite thick casts may, as a result of their elasticity, become sufficiently plastic to pass narrower tubules. Also the occasionally peculiar

tortuous or compressed, accordionlike forms must in no instance be looked upon as derived from the convoluted tubules. These forms are, perhaps, produced by the cast meeting with some obstruction and being synchronously subjected to strong pressure from behind (Fig. 115, *g*).

As to the **origin of casts**, clinical and pathologico-anatomic, as well as experimental, researches have thus far offered no generally adequate conception. The most probable assumption is that they are *albuminous derivatives* and sometimes formed as a result of transformation and coalescence of renal tubular epithelia, sometimes by these and the leucocytes together, or, finally, that they originate by a coagulation of the proteid bodies in the renal tubules similar to coagulation of the blood-coloring matter. That the simple presence of albumin does *not* suffice for the formation of casts is shown by the fact that, for example, in *chyluria* casts never occur. It is, therefore, probable that, in addition to albumin, a *participation of the epithelia* is essential (Senator).

Cylindroids.—While true urinary casts are only occasionally bifurcated or branched, facetted and fibrillated at the ends, and always present an *unmistakably rounded form*, there are occasionally observed *flattened, ribbonlike* formations which, because of a certain similarity to casts, are deserving of mention. These, also, may be studded with many forms of finely granular elements. They are most frequently observed in cholera, scarlatina, recurrent fever, and pyelitis. That they originate in the kidney appears to be extremely improbable.

5. Pus.—Pus-corpuscles are characterized by their granular and often fatty-degenerated protoplasm and the presence of *several* (from two to five) *polymorphous nuclei*. Their occurrence in *large numbers* (microscopic) indicates a probable suppuration, the existence of which can usually be suspected by the macroscopic appearance of the urine. The seat of the suppuration must be determined by a study of the other formed elements. The shape of the pus-corpuscles is of no especial significance. Many observers state that the pus-cells often show numerous prolongations [ameboid distortion] in chronic pyelitis (?) [page 367]. According to von Dittel, the urine of cystitis becomes neutral or alkaline in a few hours, while in *affections of the renal pelvis* and *kidney* the acid reaction persists *for days*.

In ammoniacal fermentation a peculiar mucilaginous liquefaction of the pus takes place (see No. 6), in which, at most, only a few nuclei can be seen on microscopic examination in addition to certain crystalline elements which will be spoken of below.

6. Mucus.—In the cloud ("nebecula") to be seen even in normal urine nothing is found except a few squamous epithelia and bacteria. The same elements occur in a transparent, faintly fibrillated matrix in mild vesical irritation. On addition of dilute acetic acid slight, but distinct, cloudiness appears.

The formation of mucus in chronic cystitis (and in the rare putrid form) is of much more importance. *In this condition a rapid disintegration of the pus-corpuscles even in the bladder occurs under the influence of ammonium carbonate and a gummy or honeylike mucous formation can be observed.* According to the investigations of A. Kossel, this is caused by swelling and solution of the pus-cell nuclei under the action of the sodium chlorid and ammonium carbonate present in the urine *(nuclein-mucus).*

See also gonorrheal shreds and spermatorrhea (page 371).

7. Fibrin is readily recognized by the distinct fibrin network which has already repeatedly been spoken of in connection with other conditions. The fibrin fibrillæ are most beautifully shown in the fortunately rare croupous coagula which are voided after the use of too strong urethral injections.

8. Fat occurs partly inclosed in granular cells, partly free, and is easily recognized by its well-known optic and chemic peculiarities. Sometimes it is observed in the form of innumerable minute globules, sometimes in large drops, especially in the large white kidney. Large numbers of small and large fat-droplets are almost constantly found in chylous urine.

9. Seminal constituents are observed especially in morning urine when discharge of semen has occurred as a result of spontaneous emission or of onanism or coitus. The spermatozoa often occur in the form of a quite dense white cloud flecked with small, glistening puncta, and usually show certain variations in shape. In micturition spermatorrhea, the spermatozoa, according to Fürbringer, show peculiar "*ruffles adhering to the head,*" which may be looked upon as membranous remnants indicating incomplete development of the spermatozoa. In the author's experience, this phenomenon is very rare.

10. Pigment.—*(a)* Derived from *blood-coloring matter,* it occurs usually amorphous or as fine and coarse granules, free or

inclosed in cells. It is much less often observed in the form of hematoïdin crystals and needles. (The latter have been observed by the author in only a few instances, viz.: after severe acute hemorrhagic nephritis and in amyloid kidney.) It occurs abundantly in small and large clumps, or in slender and thick cylinders, in hemoglobinuria (see page 303).

This pigment is distinguished from bilirubin, which is much rarer, by its insolubility in potassium hydrate.

Blood-corpuscle *detritus* in the form of droplets, clods, and pigment casts occurs in hemoglobinuria (see page 304).

(b) Melanin appears as brown or deep black, finally granular pigment, free and inclosed in leucocytes.

(c) Indigo ("urine-blue") sometimes forms delicate, light- and dark-blue needles, which are usually arranged in stellate form (see page 308).

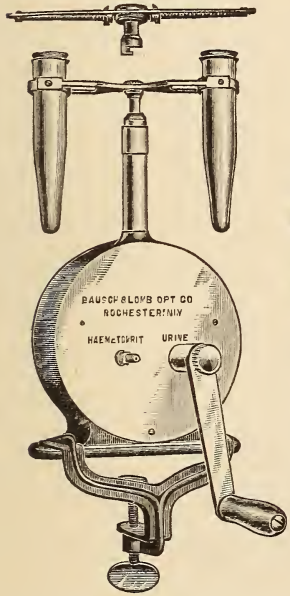
11. Tissue-shreds in Tuberculosis.—In the purulent or bloody-purulent sediment (of *acid* urine) in urogenital tuberculosis there are not infrequently seen with the naked eye rounded or shredlike, somewhat ragged, flocculi, about the size of the head of a pin, which on microscopic examination show, in addition to pus-cells, chiefly fatty *detritus*. After specific staining with Koch's stain these can be recognized as dense collections of tubercle bacilli.

12. Tissue and Neoplasm Elements.—In acute septic cystitis small and large shreds of mucous membrane are now and then voided with the urine. More frequently, however, the necrotic tissue undergoes rapid disintegration.

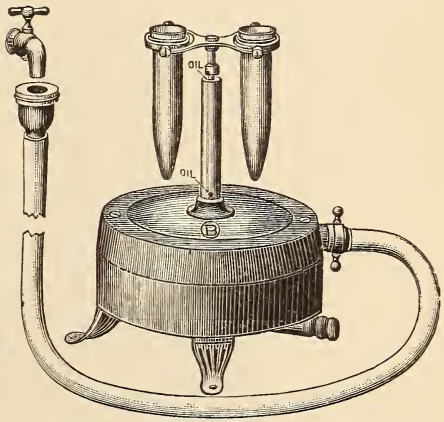
As a rule, portions of neoplasms are but seldom voided. They are most frequently observed in cases of *villous tumors of the bladder* after a catheter has been moved about several times in the bladder. Under these circumstances not only multilamellated epithelia in large amount can be found, but also distinct villi covered by thick layers of epithelia. In several instances the author was able to obtain in this manner fresh fragments of villous tissue which established a diagnosis.

Spontaneously exfoliated fragments of tumor are not infrequently voided with the urine after they have become more or less incrusted. Under such circumstances their characteristic features are obscured.

We cannot too emphatically warn against a diagnosis based upon individual *cancer-cells*. All experienced observers who are accustomed to control their cancer diagnosis by necropsies agree that the diagnosis of cancer can never be based upon the appearance of so-called *polymorphous epithelial* cells. *Of great value, however, is the numerous presence of epithelial elements in connection with oft-repeated hemorrhages*—in the absence of serious signs of cystitis (pus, etc.). The numerous occurrence of *fat-*



[Fig. 119.—Hand Centrifuge, Double Geared.]



[Fig. 120.—Water-Motor Centrifuge.]

granule spherules may also occasionally be of diagnostic value, provided nephritis does not exist.

The author observed two such cases: in one, which was a carcinoma of the left kidney, small, wormlike blood-coagula were voided several times, which directed attention to renal hemorrhage. As numerous fat-granule corpuscles were also present and all signs of nephritis absent, he no longer doubted the diagnosis of carcinoma, which was confirmed at necropsy. No tumor could be palpated.

13. Parasites.—*(a) Vegetable.*—In addition to numerous cocci and bacilli, which are particularly numerous in ammoniacal urine and are

designated as *micrococcus* and *bacterium ureæ*, there occasionally occur *sarcinæ*, *leptothrix*, and *yeast-cells*, the latter especially in diabetic urine, without their presence being of any especial interest. Much less frequently are found the threads and spores of *soor* [*thrush fungus*], which may be washed away by the urine from their rare location in the vagina.

Of *pathogenic* bacteria, the staphylococcus (in renal abscess), the streptococcus and gonococcus, the tubercle and typhoid bacilli, recurrens spirilla (Gräber), and actinomyces are observed in the urine.

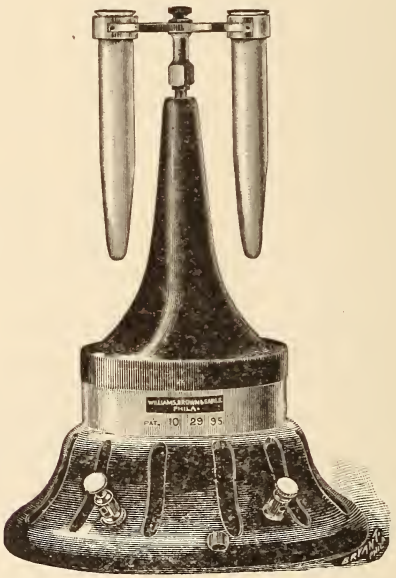
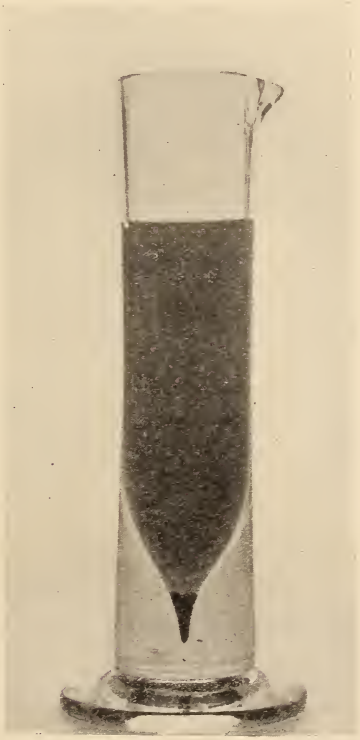


Fig. 121.—Purdy's Electric Centrifuge. (One-fourth Actual Size.)

Diagnostic interest is thus far confined especially to the detection of **gonococci, tubercle bacilli**, and **actinomyces** elements. [The typhoid, proteus, and colon bacillus and the streptococcus are also frequently found in suppurative conditions of the urinary tract. Indeed, the colon bacillus is by many considered the most frequent exciter of cystitis, pyelitis, and pyelonephritis (in about two-thirds of the cases), and it has repeatedly been found in purulent nephritis, prostatitis, paranephritis, and allied affections. The urine of uncomplicated colicystitis is almost invariably acid. Ammoniacal cystitis is due to mixed

infection or to the relatively rare exclusive presence of other species of bacteria, especially the proteus Hauser or staphylococcus. It is important to remember that the colon bacillus is not infrequently found upon the preputium or vulva, and that catheterization may transport it to the bladder; and, furthermore, that in the incontinence of retention the urine may become infected from the urethra.—Brooks.]



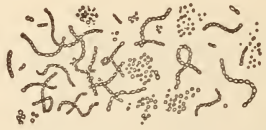
[Fig. 122.—Urine Cylinder for Collection of Sediment.]

As regards the gonococcus we have already stated in part the chief features, and will give below supplementary remarks in connection with the consideration of gonorrhea. By the demonstration of tubercle bacilli the existence of urogenital tuberculosis is established. Attention should be directed especially to small, crumblike admixtures in the purulent sediment of the bloody or purulent urine. Now and then gonococci and tubercle bacilli are found associated. In such cases the gonorrheal infec-

tion appears to have furnished a favorable soil for the tuberculous process (Stinzing).

In the diagnosis of tubercle bacilli in the urine the greatest care should be observed. The mistaking of *smegma bacilli* for tubercle bacilli has, in this respect, repeatedly led to error and serious consequences (extirpation of healthy kidneys!).

Whenever it is possible, the urine should be secured, especially in women, with a sterile catheter after thorough cleansing of the urethral opening. If this is not practicable, careful decoloration [of the prepara-

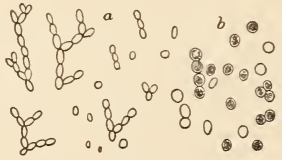


[Fig. 123.—Micrococcus Ureæ. × 500. (After *von Jaksch.*)]

tion] for at least *one hour* in *alcohol* is necessary. Under certain circumstances animal experiments are required to decide the diagnosis.

Actinomycotic rosettes are usually of much rarer occurrence in the urine than in the sputum and stools. Here, also, they appear in the form of small, yellow, sandlike granules.

For the demonstration of bacteria in the urine *centrifugation* of perfectly fresh urine is of the greatest value. Gärtner's spinning centrifuge, made by Hugershoff, of Leipzig, appears to the author to be both practical and cheap. It is also adapted



[Fig. 124.—Yeast Fungus. (After *Harley.*)]

for the volumetric estimation and counting of the red blood-corpuscles. The author has used it for years in innumerable examinations with entire satisfaction. Krefting's centrifuge, made by Gallas, of Christiana, is less expensive, and can be recommended for physicians' use. This apparatus also is in daily use by the author. [The Bausch & Lomb Optical Company make a reliable and most serviceable hand centrifuge (Fig. 119) which meets perfectly all requirements. The water-motor centrifuge (Fig. 120) is used to a very large extent. It runs very

smoothly and rapidly enough for all practical purposes and can be connected with any faucet. The best centrifugal apparatus is the electric (Fig. 121). With such an instrument almost any desired speed can be obtained.—Brooks.] If for any reason centrifugation cannot be done, sedimentation in a conic glass is necessary. [The accompanying illustration (Fig. 122) shows the form of urine glass, made by Whitall, Tatum & Co., now in use in the Post-graduate Laboratory to the exclusion of all others. These cylinders are made of very thick glass, measure nine inches in height and two inches in width, and weigh one and one-fourth

Fig. 125.—Urate of Soda and Crystals of Uric Acid *(h)*, Oxalate of Lime *(o)*, and Cystin *(c)*. × 350.

pounds. The great weight and broad base prevent tilting. They have given perfect satisfaction.—Brooks.] If no granula are found in the sediment, it is advisable to filter the concentrated sediment, carefully scrape off the residue with a spatula, and then gently triturate it with a glass rod (if necessary, a few drops of physiologic salt solution may be added) in a watch-glass [or any appropriate dish], and make a dry preparation from the mixture.

(b) Animal.—Echinococcus occurs but rarely in the course of the urinary apparatus. The diagnosis can be made only by the microscopic demonstration of hooklets or fragments of cyst membrane (page 390 and Figs. 141 and 142).

The ova of *distomum hæmatobium*, a worm occurring in the veins of the bladder and rectum (especially in Egypt), often enter and are passed in cloudy and bloody urine. They are distinguished by their elongated, ovoid form and spinelike prolongation at one pole. They occur most numerously in the blood-coagula (page 123 and Fig. 66).

Furthermore, the embryos of *filaria sanguinis* are also frequently observed in the chylous urine voided by inhabitants of the tropics. Here, also, the number of embryos is the greater the more richly bloody the urine (see page 114).

Oxyuris vermicularis may occasionally be found in the urine of little girls, into which the threadlike organism enters from the vulva.

[Fig. 126.—Sodium Urate Crystals. × 350. (After *Peyer.*)]

Trichomonas and *cercomonas* forms are met with now and then in the urine without any significance.

NONORGANIZED URINARY DEPOSITS.

Since a *sharp* distinction between the crystalline or amorphous elements occurring in acid or alkaline urine is impossible, the different deposits will be considered without reference to the reaction of the urine and the latter only occasionally be mentioned. The amorphous and crystalline *pigments* have already been discussed (page 343).

Acid sodium urate (Fig. 125) forms the brick-red sediment (col-

PLATE II.

[Uric-Acid Crystals. Normal Color. × 450.
(After Peyer.)]

ored by uroerythrin) constantly found in highly concentrated urine. Microscopically it is composed of densely arranged fine granules which individually do not appear to be colored. It often is freely deposited upon such morphologic elements—casts, etc.—as may be present in the urine. It disappears at once on heating or addition of dilute potassium hydrate, while HCl after a time (from ten to twenty minutes) forms with them crystals of uric acid.

Uric acid (Fig. 125, *h*, and Fig. 127, *e-h*) occurs in minute to pin-head sized, bright-red granules which are sometimes found in clear urine, but more frequently in urine containing brick-dust deposit. They are composed of densely arranged, pale or dark *yellow-colored crystals*, which occur in the form of whetstone, plate, barrel-shaped, dumb-bell, and rosette forms. They occur most frequently in the uric acid diathesis

Fig. 127.—Hippuric Acid *(a-c)*, Sodium Urate *(d)*, and Uric Acid in Whetstone, Dumb-bell, and Rod Form *(e-h)*. × 350.

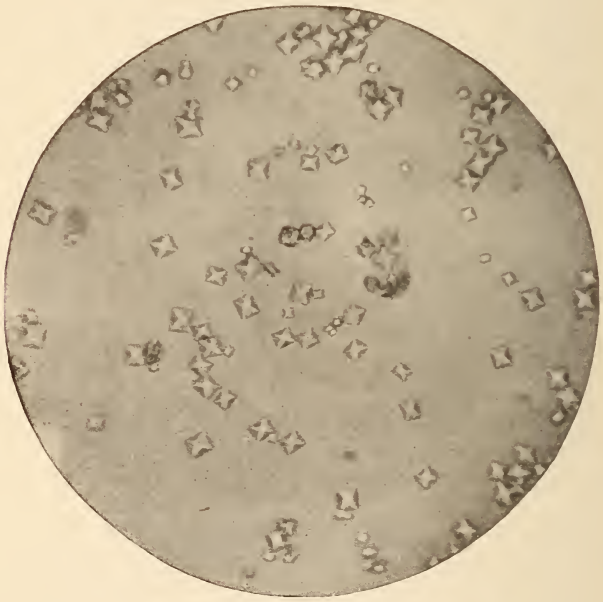
(especially *after* an attack of gout), in pernicious anemia and leukemia, and in concentrated febrile urines, excessive meat diet, etc. In perfectly normal urine uric acid is combined with bases and held in solution as neutral sodium urate.

Addition of sodium hydrate dissolves the crystals at once. Further addition of a few drops of hydrochloric acid causes them to reappear in plates and whetstone forms.

Oxalic acid (Fig. 125, *o*, and Fig. 128), which is dissolved in normal urine by acid sodium phosphate, occurs in many diseases, and *occasionally, also, without any demonstrable disturbance* (physiologic oxaluria). It appears in the very characteristic form of calcium oxalate crystals. They present the well-known envelope shape, sometimes more in the

form of pointed octahedra, and sometimes in cubic form. The envelope crystals are the most common forms [dumb-bell, ovoid, and hexagonal forms are occasionally observed—Brooks]. In addition to their occurrence in diabetes mellitus, catarrhal jaundice, and a number of other diseases, they not infrequently appear in azoöspermatorrhea and in chylous urine in filariasis. Abundant ingestion of foods containing oxalic acid (grapes, apples, oranges [rhubarb], etc.) may increase the amount of oxalic acid in the urine.

The crystals are at once dissolved by addition of hydrochloric acid, but resist acetic acid. Their occurrence is of no especial diagnostic sig-



[Fig. 128.—Calcium Oxalate Crystals in Urine. × 350.]

nificance. [Constant excess of oxalate of lime in the urine is indicative of the so-called lithic acid diathesis: *i.e.*, of suboxidation. In *excess*, it signifies excess of uric acid. In the words of Dr. Basham, which we unreservedly indorse, this phenomenon "belongs to a class of disorders characterized by malassimilation, the evidence of which is drawn, not only from the disturbances in the nervous, circulatory, and digestive organs, but from the presence in the urine of an excess of one of the most important excrementitious matters of the urine—uric acid. The causes of the disturbance are various. They may be traced chiefly to overtension of the mental faculties, to the anxieties of business or of professional life, coupled, perhaps, with errors of diet and neglect in

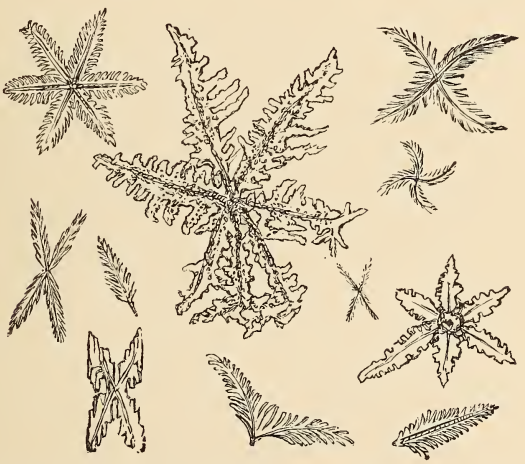
PLATE III.

[Uric-Acid Crystals with Amorphous Urates. × 450.
(After Peyer.)]

many minor matters of the general health." Indican is very commonly present in these cases.—BROOKS.]

Hippuric acid (Fig. 127, *a-c*) rarely occurs in normal urine. It is most frequently observed after employment of salicylic acid. Otherwise it is also seen in diabetes mellitus and many hepatic disturbances. It appears in the form of needles and rhombic prisms, which in contrast to triple phosphate crystals resembling them, are *insoluble in acetic acid.*

Cystin (Fig. 125, *c*) is occasionally observed in periodic cystinuria of otherwise healthy subjects, and also in articular rheumatism. It occurs in the form of pale, six-sided plates, which may be mistaken for uric acid. They are distinguishable from the latter by the fact that they are *dissolved by addition of a few drops of ammonia.*



[Fig. 129.—Feathery Crystals of Triple Phosphate. × 350. (After *Tyson.*)]

Leucin and **tyrosin** (Fig. 93; see page 212) can usually be demonstrated in the sediment only after gradual evaporation or vaporization of a small amount upon a slide. The *leucin spherules* may occasionally *be mistaken for ammonium urate.* From the latter, however, can be obtained by addition of HCl the above-described crystals of free uric acid.

Leucin and tyrosin have more frequently been found in acute *yellow* atrophy of the liver, and less frequently in phosphorus poisoning and a few acute infectious and chronic blood diseases.

Cholesterin (Fig. 91) occasionally appears in the urine (in filaria sanguinis, echinococcus, etc.).

Fat-needles and small fat-crystal rosettes are occasionally observed in the urine in "large white kidney." They can be positively identified by the reactions already referred to (page 209).

Crystals of **triple phosphate** (Fig. 130, *t*)—*i.e.*, ammonic-magnesian phosphate—are most frequently met with in *faintly acid and alkaline urine.* They appear chiefly in the form of three-, four-, or six- sided prisms with oblique ends, and are then designated as "coffin-lid" crystals [resembling the lid of a German coffin—BROOKS]. In addition to this form the less developed "sledge" form is quite often observed. They are distinguished from the crystals of oxalic and hippuric acid by their ready solubility in acetic acid. In ammoniacal fermentation (chronic cystitis) they are never lacking. Associated with them are met the yellow or brownish spherules of **ammonium urate** (Fig. 130, *a*). These are usually collected in small groups, and are not infrequently provided with numerous spicula, which give them the appearance of a *thorn-apple.* They are distinguished

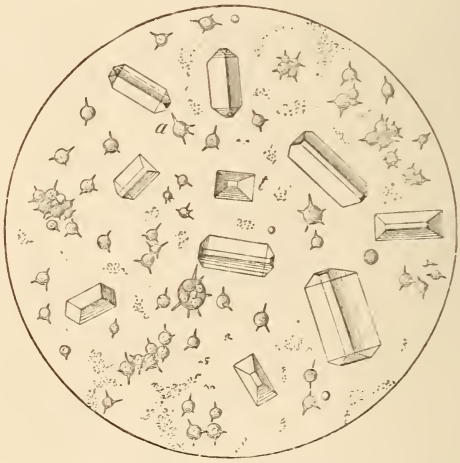


Fig. 130.—Crystals of Triple Phosphate *(t)* and Ammonium Urate *(a).* × 350.

from leucin by their reaction with hydrochloric acid, as already stated, and their solubility in potassium hydrate.

Calcium carbonate (Fig. 131, *a*) occurs in similar, but *much smaller, spheres* than ammonium urate. Sometimes they lie in pairs in biscuit or dumb-bell form, sometimes in groups of four, six, or more. On addition of hydrochloric acid rapid solution of the crystals occurs with active liberation of CO_2.

It occurs in an amorphous form, with, as well as without, the crystals, and is found in faintly acid, alkaline, and ammoniacal urine.

Calcium sulphate (Fig. 131, *b*) occurs in the form of long, colorless needles or rods, which are insoluble in acids and ammonia. It is seldom observed.

PLATE IV.

[Sediment of Alkaline Fermentation. × 450. (After Hoffman and Ultzmann.)]

Neutral calcium phosphate (Fig. 131, *c*) is found sometimes in faintly acid, sometimes in distinctly alkaline, urine. It occurs in the form of pointed wedge-shaped prisms, which appear singly or arranged in rosettes. They disappear on addition of acetic acid.

Magnesium phosphate (Fig. 131, *d*) forms quite large rhombic plates, which are readily soluble in acetic acid. It is found in part in the white or whitish-yellow sediment which not infrequently forms abundantly in the urine of neurasthenics. *It can be obtained in very large thin plates from the surface of many urines which present a delicate, glistening iridescent surface* (Fig. 133). These plates may be secured for examination by holding a cover-glass in a pair of forceps in such

Fig. 131. (After *von Jaksch.*)

a, Calcium carbonate. *b*, Calcium sulphate. *c*, Neutral calcium phosphate. *d*, Basic magnesia phosphate. × 350.

a manner that the whole surface of the glass comes in contact with the urine surface, and then placing on a glass slide. The thin plates, with their many sharp angles, resemble fragments of broken window-glass.

The *phosphates* occur more frequently in amorphous than in crystalline form. They appear as small, colorless granules which are soluble in acetic acid. The similar urate sediment which may be mistaken for phosphates forms uric acid crystals on addition of acetic acid.

The morphous and crystalline phosphates often occur together in faintly acid or alkaline urine; on the other hand, the crystalline forms are *never* observed in ammoniacal fermentation.

SPECTROSCOPY OF THE URINE.

With the "pocket spectroscope" (Fig. 69) the following substances can usually readily be distinguished (cloudy urine must be diluted with water) :—

1. Oxyhemoglobin, characterized by the well-known bands (page 130), is found in *freshly voided,* bloody urine containing well-preserved (hematuria) or no red blood-corpuscles (hemoglobinuria).

[Fig. 132.—Calcium Phosphate Crystals. × 350. (After *Peyer.*)]

2. Methemoglobin, characterized especially by the bands in red :—

(a) In old, long-standing urine in hematuria.

(b) In freshly voided urine in methemoglobinuria after poisoning with chlorate salts, anilin bodies, etc.

3. Urobilin is recognized by a band lying between green and blue.

4. Hematoporphyrin, almost exclusively after poisoning with *sulphonal* and *trional;* recently, however, also observed by Fränkel and Sobernheim in a typhus patient at the acme of the

fever and during convalescence. [It may rarely be present in normal urine.—Brooks.]

The urine is dark bluish red, almost opaque, but gradually clears up. No abnormal elements are found *microscopically*. If the urine is heated, no distinct change occurs, and just as little on addition of HCl. A yellow color is produced by the addition of ammonia. On boiling with nitric acid it becomes pale (bleached).

Spectroscopically there are found two absorption bands in yellow and light green and darkening of the whole right portion of the spectrum, beginning with green-blue. Liquor ammonia sulphurati produces no

Fig. 133.—Magnesia Phosphate from a Fresh Pellicle upon the Surface of Urine.

alteration. On the other hand, addition of HCl causes the appearance of two bands, of which the first is smaller and less distinct and lies at the junction of orange and yellow, and the second in yellow and green. Addition of ammonia causes the first-named two bands to reappear.

It may further be remarked that, after *sulphonal poisoning*, a genuine (toxic) nephritis *without* hematoporphyrin may also occur, as has been observed by Stern and the author.

The demonstration of hematoporphyrin, *aferrous hematin* (Hoppe-Seyler, Nencki, and others), in the form of crystallized combinations is possible (Fränkel).

CHARACTER OF THE URINE IN CERTAIN DISEASES.

I. DISEASES OF THE KIDNEYS.

1. Acute Nephritis.[1]

It is most convenient to distinguish an *acute hemorrhagic* and a *nonhemorrhagic* form according to the bloody or nonbloody character of the urine.

In both conditions the quantity of urine voided is more or less considerably diminished,—to from 500 to 1000 cubic centimeters in twenty-four hours,—or there is transitory anuria. Sometimes the oliguria persists only for a short time; not infrequently, however, for weeks. With subsidence of the inflammatory process, increase in the quantity of urine gradually or rapidly begins; the latter is the case particularly in absorption of the not rarely intense hydropsic accumulations. In mild cases the diminution of the urine is slight.

The specific gravity is very variable. In the hemorrhagic form it is usually between 1.010 and 1.015, but sometimes much higher; in the nonhemorrhagic form it is usually raised to 1.025 or 1.030. In both varieties the urine is strongly clouded by admixture of morphologic elements.

The color of the bloody urine is light flesh-water to dark beer-brown, according to the amount of blood and the concentration. In not too dark urine distinct dichroism is observed. In patients confined to bed the night urine is always less bloody than the day urine. In the second form the urine is light or dark yellow in color. Both varieties show a more or less abundant sediment, which in the first form is dark brownish red, in the second pale yellow.

The albumin content is usually more abundant in the nonhemorrhagic than in the hemorrhagic form; the latter not infrequently gives only a slight precipitate. The total loss of albumin in twenty-four hours averages from 5 to 8 grams, but may reach 20 grams or more. Urea, uric acid, and chlorids are usually diminished.

Microscopically there are found in the hemorrhagic form numerous red blood-corpuscles, partly isolated, partly in clumps

[1] See Figs. 114 and 115.

PLATE V.

[Ammonium Urate, showing Spherules and Thorn-apple-shaped Crystals. × 450. (After Peyer.)]

or adhering to casts. These sometimes show no alteration, either in form or size; more frequently, however, they appear smaller than normal. Very often, especially when the disease has existed for a number of days or weeks, there are seen in addition to unaltered red cells delicate, transparent discs with sharp, circular outline; these are the "leached" red blood-cells, or "blood-rings." Almost invariably there are also observed "red blood-corpuscle casts," composed of densely collected erythrocytes or blood-rings; finally, leucocytes, usually in moderate numbers. More or less numerous hyaline and granular casts are seen whose granulations are composed partially of albumin granules or leucocytes, partially of epithelia from the renal tubules. The latter also occur free or in groups, in greater or less numbers, and after the disease has lasted for some time are in a state of partially fatty metamorphosis.

In the sediment of the *nonhemorrhagic* form isolated red blood-corpuscles occur, if at all, while the leucocytes are always very numerous. Epithelia are not infrequently found in large numbers, and also as "epithelial tubular formations," though less often than in the above-mentioned variety. They are frequently in a state of advanced fatty degeneration, however, even after the disease has lasted for a few days.

The division of morbus Brightii into two subvarieties as here given is justified, first of all, by the anatomic picture.

In the first form the kidneys are always enlarged, often to a considerable degree, and numerous hemorrhages are present upon the outer and cut surfaces. The inflammatory phenomena involve the glomeruli as well as the renal tubuli. In the second form the kidneys are usually only moderately enlarged and free from hemorrhages. The disturbance affects chiefly the renal tubules. Anemia of the organ and albuminous swelling and fatty metamorphosis of the renal epithelia characterize this form.

Many transition forms, both clinico-microscopically and pathologico-anatomically, are not infrequently observed.

In order to determine whether an acute nephritis or an intercurrent aggravation of a pre-existing chronic affection is present, the other clinical findings, as well as the anamnesic statements, must first be considered. The latter condition must be thought of especially when, in spite of the apparent freshness of the affection, the amount of urine is quite abundant or even increased, the specific gravity low, and red blood-corpuscles and casts are found in the scanty sediment.

Etiologic differences for both of these forms of acute Bright's disease should also be considered.

As is known, the disease very seldom occurs primarily. It appears frequently as a secondary affection, especially under the influence of infectious and toxic agents. Experience has shown that in *one* series chiefly acute hemorrhagic, in another principally nonhemorrhagic, nephritis occurs. As a consequence of typhoid fever, croupous pneumonia, severe "colds," in sepsis and after inunctions with petroleum, naphtol, etc., the author almost constantly observed the acute hemorrhagic form, while in diphtheria and pregnancy nonhemorrhagic nephritis occurred. This form of nephritis is also of constant occurrence in cholera, in which numerous fatty epithelia and distinct fat-granule cells appear in the urine with surprising rapidity.

In scarlatina the author saw chiefly the second form, although urine with decidedly bloody character was not rarely observed; the "*genius epidemicus*" appeared to play an important rôle in this respect. Anatomically, the scarlatinal kidney almost always presents the appearance of the "large white kidney."

"*Bright's disease of pregnancy*," to which von Leyden especially has assigned a prominent position, also manifests in the great majority of instances the features of the pale fatty kidney. The urine is scanty, usually highly albuminous, and contains numerous granular casts with fat-droplets and not rarely fat-granule cells. Red blood-cells and even hematoïdin crystals also occur, although the latter are very rare.

2. Chronic Nephritis.

(a) Diffuse Nephritis (Large White Kidney[1]).—This form *occurs but rarely in practice,* and can usually be diagnosticated with certainty; careful examination of the urine contributes to this end in a high degree, and even alone often suffices for the recognition of the disease.

The *urine* is pale yellow, always cloudy, and not infrequently shows upon its surface a somewhat fatty gloss. Usually an abundant sediment is precipitated. The *quantity is always diminished,* only seldom on certain days somewhat more abundant, averaging from 300 to 600 cubic centimeters and toward the end of life usually reduced to from 200 to 100 cubic centimeters.

The *specific gravity* is almost always increased, inversely proportionate to the quantity. As a rule, it is between 1.020

[1] See Fig. 115.

PLATE VI.

[Crystals of Phenylglucosazone. × 450.
(After v. Jaksch.)]

and 1.030. The *albumin content* is always very high; often more than the half of the volume of urine examined coagulates on heating.

Microscopically there are constantly found very numerous formed elements. These are composed of numerous leucocytes, a very scanty number of erythrocytes, and usually extremely numerous casts of various widths and lengths, not infrequently presenting notches, facets, convolutions, swellings, etc. In most of them can be seen fine or coarse granular fat and numerous fat-granule cells. The latter are also quite often observed free in the field of the microscope, and undoubtedly correspond to fatty-degenerated renal epithelia.

If these findings are observed almost unaltered at a number of examinations, the diagnosis of large white kidney can be made with the greatest probability, particularly if the signs of cardiac hypertrophy are absent and hydrops (usually quite marked) is present. The character of the urine is explained by the intense fatty degeneration of renal epithelia, the anemia, and the diminution in size or complete atrophy of the majority or all of the glomerular convolutions, all of which are constantly present in this renal affection.

Severe colds, syphilis, malaria, scarlatina, etc., are etiologic factors.

(b) Chronic Nephritis (with Cardiac Hypertrophy).

1. The Ordinary Chronic Bright's Disease.[1]—This suborder is the variety *most frequently met with in practice,* and can be recognized with quite a degree of certainty by the character of the urine.

The urine is generally cloudy, pale yellow, and, as a rule, deposits only a scanty, faint sediment. It is usually voided in larger quantity than in the first-described form, averaging between 800 and 1400 cubic centimeters. The quantity seldom remains for any length of time at 600 or less. The specific gravity is variable, most frequently somewhat reduced—1.012 and 1.010. On the other hand, the albumin content is always considerable.

Microscopically there are usually found, in addition to occa-

[1] See Fig. 117.

sional red and somewhat more numerous colorless blood-cells, quite a number of more or less fatty hyaline casts, to which occasionally adhere characteristic, moderately fatty renal epithelia which are often transformed into fat-granule cells. Not infrequently these are also observed free in the urine.

The characters of the urine are fully explained by the anatomic findings, which, in addition to atrophic foci of various extent, reveal in the normal sized, rarely moderately enlarged, occasionally somewhat shrunken kidneys, numerous *fresh parenchymatous and interstitial inflammation.* The quantity of urine is more or less diminished according to the preponderance of the parenchymatous inflammation. On the other hand, it is increased when there is a marked tendency to transition into the contracted kidney.

Even under these conditions, however, which have led to the development of the features of **secondary contracted kidney,** the amount of albumin will always insure the diagnosis, in spite of the fact that the quantity of urine is augmented to 2000 cubic centimeters or more. This, however, is explained by the *constant* participation of the parenchymatous inflammatory processes, as indicated by the fat-granule cells, and the numerous casts. If the formed elements are entirely lacking for weeks at a time, the diagnosis of genuine contracted kidney may wrongly be made. The fact that the specific gravity, even in spite of the increased amount of urine, reaches 1.012 to 1.018 and the albumin content is usually high, generally protects against this error. Furthermore, careful sedimentation or centrifugation usually permits the recognition of characteristic formed elements.

2. Chronic Hemorrhagic Nephritis (Weigert's Red or Mottled Kidney).—In this renal affection, *which in acute exacerbations not infrequently gives rise to confusion with acute hemorrhagic nephritis,* the urine generally presents the character of the urine observed in contracted kidney. For this reason, therefore, it requires especial mention, since without demonstrable cause it shows a distinctly bloody character of variable frequency and intensity,—a feature which is observed in genuine contracted kidney *only* in a very small proportion of cases.

The urine is often pale yellow, and deposits only a scanty, pale-yellow or reddish sediment, or only a somewhat more dis-

tinct "nebecula," in which a few isolated red blood-corpuscles, more frequently blood-rings ("shadows"), and a small number of hyaline casts occupied by blood-rings are always found. The quantity is normal or increased—to from 1500 to 2000 cubic centimeters. The amount of albumin is slight.

Another picture is presented by the urine at the time of hemorrhage, which sometimes recurs quickly or is marked by an interval of many months. The brownish-red or intensely-dirty-brown urine often deposits an abundant sediment. Its upper layer is then from light to dark flesh-water colored. The quantity declines to 1000 cubic centimeters or less, the albumin content remains moderate, and the specific gravity is about 1.010. On the other hand, there are found microscopically numerous casts which are often so thickly covered with red blood-corpuscles and "blood-rings" that they may justly be designated as blood-corpuscle casts. Also, as a rule, numerous renal epithelia are observed free and upon casts. Fat-granule cells are never seen.

3. Contracted Kidney [Chronic Interstitial Nephritis].—The urine is pale yellow or pale green and perfectly clear. It deposits only a very scanty sediment, which may, however, be entirely absent for weeks. The quantity is usually increased to from 2 to 3 liters and may rise to from 6 to 8 liters in twenty-four hours. The specific gravity seldom attains 1.010, and usually averages 1.005. Aside from intercurrent disturbances,—uremia, etc.,—the albumin content is always very slight, and *may be entirely absent for days and weeks.* The specific gravity and the albumin excretion are less in night urine than in day urine.

Microscopically often no formed elements except isolated leucocytes and occasional hyaline casts are found even after weeks of examination with most careful illumination. If, however, the urine is examined during occasional disturbances of general well-being, especially during and after a uremic attack, casts and particularly also epithelia will not rarely be found.

It should be especially emphasized that in intercurrent febrile diseases, such as pneumonia and the like, contrary to what is ordinarily observed, no concentrated "febrile urine" is seen, but usually a pale-yellow urine with low specific gravity (Traube, Wagner).

The features of the urine are usually so characteristic that, with consideration of the other clinical phenomena, especially those manifested by the heart, the diagnosis can be made with certainty. Indeed, confusion only with amyloid kidney can occur.

The large amount of urine voided in contracted kidney is furnished by the still preserved and comparatively strongly hypertrophied parts of the kidney, especially the epithelia and glomeruli. The latter form the "granula" upon the nodular surface of the kidney. The sunken parts correspond to the atrophic parenchymatous areas, the function of which has been entirely abolished. If numerous casts are found in the urine, it may be assumed that a part of the kidney is again progressing toward atrophy, and, although restricted in its function, still secretes urine, but, owing to the more or less advanced renal atrophy, allows albumin to transude.

4. Amyloid Kidney is not infrequently mistaken for contracted kidney, because a very abundant, pale, clear, and slightly albuminous urine may also be secreted in amyloid degeneration. Aside from the fact that amyloid kidney almost always develops in pulmonary and intestinal tuberculosis, chronic bone and joint suppurations, syphilis, bronchiectasis, etc., the urine usually presents positive points of difference.

In the majority of cases the quantity of urine is *diminished* to from 1000 to 600 cubic centimeters and the specific gravity averages 1.015 to 1.020, but often still higher. In addition, the albumin content is abundant, and a marked, though scanty, sediment is present.

Microscopically it is usually characterized by its often rich content of long hyaline casts, which are often two or three times as long as the diameter of the field of the microscope, and usually slender. In the last days of life the number of casts is often considerably increased, and unusually long and very broad forms appear. Red blood-corpuscles are seldom observed, but colorless corpuscles are quite frequent *(the author several times observed a large number of them covered with long, delicate hematoïdin needles)*. As a rule, epithelia are but seldom met with. The author has never observed fat-granule cells (although he has followed more than thirty cases to necropsy).

Contracted amyloid kidney should be suspected especially in syphilitic subjects.

Contusions of the Kidney often give rise to hematuria lasting for days or for from three to four weeks. Occasionally the hematuria may not occur until one or two days after the injury, and recur at irregular intervals.

Hydropyonephrosis is not infrequently attended by a decided intermittence in the amount of urine, since obstruction of the ureter may cause arrest of the flow of urine, and, on the other hand, reopening of the passage produces a great and rapid increase. However, a diagnosis can never be based upon the character of the urine alone.

Rupture of Abscesses of the Kidney leads to the appearance of more or less considerable amounts of pus in the urine.

Malignant Neoplasms of the Kidney give rise to *hematuria* in somewhat more than half of the cases. Portions of tumor are found in the urine only in extremely rare instances (see page 374).

II. DISEASES OF THE URINARY PASSAGES.

Concretions in the Pelvis of the Kidney not infrequently produce an acute irritation (with colic), which sometimes occurs *without,* sometimes with, *changes in the urine.* The latter chiefly consist of *bloody* admixtures. If the amount of blood present is large, the question can at once be decided by the unaided eye; it is otherwise when the urine is macroscopically unaltered and the chemic tests for blood give negative results.

In all instances in which the diagnosis of renal colic due to stone is entertained, careful microscopic examination of even apparently perfectly normal urine should always be made. If red blood-corpuscles, sometimes in small groups, are found in the sediment (under certain circumstances after centrifugation), this may be of great value. In addition to blood-cells, various crystals are frequently met with: urate, oxalate, or phosphate of lime.

As a rule, **pyelitis** can be diagnosticated with a certain degree of probability from the character of the urine only in the chronic form.

The urine is usually pale yellow and cloudy, and deposits a fine, flocculent, purulent sediment in which microscopically small plugs ("pus-casts") not infrequently can be found. These come from the papillary portion of the kidney. If renal irritation coexists, hyaline and granular casts are seldom absent.

The quantity of urine is almost always increased to from 3 to 4 and more liters; its specific gravity is reduced to from 1.008 to 1.010. Albuminuria is not infrequent. Hemorrhages and discharge of cylindric blood-coagula, from 5 to 6 millimeters thick and 8 to 12 centimeters long, often occur.

In the so-called "**cystic kidney**" the urine not infrequently presents a quite similar character; it may, however, resemble more closely the urine of contracted kidney, owing to the fact that pus may be absent for a long time and that other morphologic elements rarely occur.

Cystitis.—In mild vesical irritation or mucous catarrh the urine is generally faintly acid, nonalbuminous, and pale yellow in color. It deposits a scanty nebulous sediment which microscopically shows only a slightly increased number of bladder epithelia and leucocytes. In purulent catarrh, also, the findings are usually similar. The sediment is finely flocculent in acid urine, and greenish and slimy in alkaline urine. The formed elements are usually altered, and a slight *albuminous* cloudiness is also observed.

Ammoniacal urine presents a repulsive odor, a dirty-brownish color, and a thick, mucilaginous sediment, which is formed by decomposition of the pus-corpuscles caused by the action of ammonium carbonate. It contains chiefly large numbers of bacteria and triple phosphate crystals.

The decomposition of urea into ammonium carbonate occurs either in the bladder or shortly after voiding. It always takes place under the influence of certain microbes, among which, according to Schnitzler, the *proteus vulgaris Hauser* is most frequently found. It can split up urea and produce ammoniacal fermentation. It occurs in pure culture not only in the bladder, but also in the renal pelvis. The equally important *bacterium coli* is less infectious (page 78). Vesical catarrh [cystitis] is caused either indirectly by the decomposed urine (Rovsing) or directly by pyogenic bacteria—probably by both influences (Schnitzler).

In *tuberculous* cystitis the purulent urine has a distinctly acid reaction.

[The Differential Diagnosis of Pyelitis and Cystitis.—G. Rosenfeld states that in pyelitis the urine is almost always acid. Without medicinal action he has never seen pyelitic urine otherwise than acid. It is important, however, to employ fresh urine for determining the reaction. An axiom, he says, which we can make use of in diagnosis is: *Nonacid urine does*

not belong to an uncomplicated pyelitis. In many forms of cystitis the urine is usually acid—*e.g.* in gonorrheal and tubercular cystitis and in cystitis of uric acid stone,—but, since we do not consider the acid reaction in a positive sense, this is of no especial significance.

As to the *arrangement of the pus-corpuscles,* they do not offer many variations: they are either single, in shreds, or collected in spheric masses. The spheric masses, which consist only of pus-corpuscles, are of no diagnostic significance; they accompany any suppuration of a cavity connected with the uropoietic system. The shreds, which, as universally accepted, originate from the urethra, indicate that the pyuric process is located either in the urethra or is complicated with such. This, of course, points rather to a cystitis than to a pyelitis. The *form of the leucocytes* is also of great importance: they are either round, crenated, or ragged, as though fixed during ameboid movement. These ragged, nonmotile leucocytes are derived, according to Rosenfeld, from the pelvis. When this phenomenon is observed only in isolated corpuscles it is of little importance, but when in one case the whole field of the microscope is filled with quite round leucocytes, in another case full of ameboid-distorted leucocytes, the differential diagnosis is important. Hence Rosenfeld states: *Ameboid-distorted leucocytes speak in favor of pyelitis.* The finding of round forms only does not exclude pyelitis, but directs attention rather to the existence of cystitis.

The *red blood-corpuscles* also manifest certain features of diagnostic value: When they are derived from the bladder they are well preserved (except vesical tumors), while those from the renal pelvis are morphologically altered and are partly or wholly deprived of their coloring matter.

As regards the *epithelia,* only such masses as are characterized by a bright nucleus, a rounded form, and a size not much larger than a white blood-corpuscle,—and, perhaps, also one or two such epithelial cells if they are imbedded in a clump of leucocytes,—can be regarded as coming from the renal pelvis. The frequently described imbricated arrangement of the renal pelvic epithelia is never observed in the urine. The statements in this regard and in reference to the occurrence of large

squamous epithelia in cystitis are of a traditional nature and inapplicable in either instance. Large squamous cells have diagnostically nothing to do with vesical catarrh; so little, indeed, that in the presence of large numbers of them and a few white corpuscles one can safely diagnosticate leucorrhœa and urethritis, but not cystitis.

The most important of all criteria is *the ratio of albumin content to the amount of pus.* This must be determined quantitatively. In cystitis, even in maximal amounts of pus which make a sediment several centimeters in height, there is never an albumin content of more than 0.1 to 0.15 per cent. The case is quite otherwise with the urine of pyelitis. Even with an amount of pus sufficient to make a layer of but from 1 to 2 millimeters high at the bottom of the vessel, there will be found an amount of albumin equaling that observed in maximal degrees of cystitis, namely: 0.1 to 0.15 and more. In very large amounts of pus the albumin content is not often above 0.3 per cent.; but what is characteristic is the high albumin content in small and minute quantities of pus and an amount of albumin always exceeding that present in the maximal degrees of cystitis. The author offers the following scheme of four degrees to express this ratio:—

	Cystitis. Albumin (percentage).	Pyelitis. Albumin (percentage).
I. Maximum degree: Numerous pus-cells in liter glass.	0.1	0.3
II. Moderate degree: Pus-sediment about ½ centimeter high.	0.06	0.2
III. Slight degree: Pus-sediment 1 to 2 millimeters high.	Just distinctly recognizable.	0.1
IV. Minimum degree: Recognizable almost only by microscope.	Not recognizable.	Distinctly recognizable.

This table, it should be remembered, is only approximate. These rules are based upon practical experience and have been controlled and verified by clinical and anatomic observations.

Rosenfeld cites a case of a man, 37 years of age, in whom puncture of a hydronephrotic sac at laparotomy offered an opportunity to study the urine derived from the kidney. The urine secreted was acid and deposited a sediment of from 1 to 2 millimeters in height. This sediment consisted almost wholly of ameboid-distorted leucocytes and a few decolored and microcytic disintegrated red blood-cells. In this urine there was 0.175 per cent. of albumin: *i.e.*, the urine showed all of the characteristics of pyelitis as above described, namely: acid reaction, ameboid-distorted leucocytes, disintegration of the erythrocytes, and — above all — a relatively high albumin content.

To summarize: Alkaline reaction is not found in uncomplicated pyelitis. The limit of albumin content, even in maximal cystitis, is 0.1, at most 0.15 per cent.

If nearly all pus-cells are ragged in contour, this phenomenon speaks in favor of pyelitis.

If the red blood-corpuscles present are principally chemically or morphologically disintegrated, this, only in microscopic hemorrhage, and in the absence of a vesical tumor, speaks in favor of pyelitis.

The characteristic symptom for diagnosis is the relation of albumin and pus according to the above scheme.—Brooks.]

Urethritis.—Simple acute inflammations of the urethra occur almost exclusively after direct irritation, and quickly subside. The mucoid or purulent discharge mixes with the urine, and usually in the form of muco-purulent shreds, which are, as a rule, formed during the passage of the urine. Careful examination of the pus, which in such cases must, if possible, be secured in the pure state by stripping pressure upon the urethra, should be directed toward detection of gonococci. Mucoid or slightly muco-purulent shreds—which, for etiologic reasons, are designated as "*tripper-fäden*" [or "gonorrheal shreds"]—are much oftener met with, especially in men.

Clap [Gonorrhea].—In the acute infection the discharge, after it has been simple mucoid for about two or three days, becomes distinctly yellowish green, purulent, or dirty brownish red, when the inflammatory phenomena are very violent and lead to admixture of blood with the secretion. On cessation of the

inflammation the discharge again assumes a more mucoid character. For examination of the secretion a freshly expressed drop of pus is best adapted, though the latter can also be taken from the urine with a pipette. In women, in addition to the urethra, the cervix is principally affected.

Microscopically there are found in the mucoid secretion, in addition to leucocytes, different kinds of epithelium, sometimes simple squamous, sometimes more polygonal or oval varieties with tail-like processes. Fürbringer saw many peculiar hyaline epithelia which, because of their tendency to turn distinct brown with iodin, he designated as iodophilic. In the stadium blennorrhoicum pus-corpuscles almost exclusively are met with, nearly all of which are polynuclear and on staining of dry preparations are recognizable as neutrophilic leucocytes. Large *eosinophilic cells,* such as are seen in the blood in leukemia, the granules of which do not fill the cell, are almost invariably found. At every stage of virulence the characteristic diplococci [gonococci] can be demonstrated in the secretion. They are most numerous in the creamlike pus.

If all trace of discharge is absent, especially any admixture with the morning urine, and if the most careful swabbing of the urethra no longer shows a trace of secretion, then the disease may be considered entirely cured. Fortunately, this occurs in the majority of cases, and should be especially emphasized in view of the widely distributed pessimism of many physicians who deny that healing of a gonorrhea ever occurs.

It is true that in not a small number of cases gonorrhea becomes *chronic.* A cloudy-mucoid discharge persists which becomes purulent after every excess in *Baccho aut Venere,* and which usually is distinctly present only as the so-called "morning drop." In such patients, who, with inattention to their condition, may no longer be conscious of their discharge, we invariably observe the "*tripper-fäden,*" the important significance of which has been emphasized especially by Fürbringer. They are translucent, mucoid or more opaque yellow, coherent formations of variable length (the author found them as long as six centimeters), ranging in size from very fine threads to the thickness of a knitting-needle, which usually appear at the beginning, more seldom at the end, of urination and can be recognized at once as

shreds. By strong force of the urinary stream and great disturbance of the fluid, many are disintegrated and quickly reduced in size; others, again, resist even the strongest flow of urine.

It is advisable to secure them as quickly as possible from the urine with a pipette and examine without delay. *Microscopically* there will then be found, according to the yellow coloration, more or less numerous pus-corpuscles (and eosinophilic cells) in addition to variously shaped epithelia from the urinary tract. Sometimes there will be seen only *isolated* squamous epithelia; at other times these are more numerous. The author always observed numerous club-shaped and sickle-formed epithelia, not rarely collected in dense clumps and bands, with distinct, comparatively small nuclei. In addition there also occur (low and high) cylindric and goblet-cells; furthermore, now and then spermatozoa and isolated blood-cells.

It is of the greatest importance for the practitioner to examine these shreds for gonococci, and very essential to make repeated examinations of a number of them. *Indeed, they should be examined with a conscientiousness equal to that manifested in examination of the sputum for tubercle bacilli in doubtful cases.* If repeated examinations invariably give negative results as to the gonococcus, *then the virulence of such shreds is almost certainly to be excluded, and, in a given case, marriage may be permitted.*

We will here consider a series of pathologic states occurring partly in conjunction with gonorrhea, the recognition of which is decidedly facilitated by the microscope.

Spermatorrhea.—On urination and during defecation (micturition and defecation spermatorrhea) there is discharged a thin, ropy mucus which may be secured in a free state by the patient. Microscopic examination shows unmistakable presence of spermatozoa, which often manifest perfect motility. Not rarely, however, they show sluggish movements in addition to external changes. These spermatozoa (like the normal) take up the anilin dyes well. If staining is done with a dilute solution of carbol-fuchsin and afterward with methylene blue, the tail and middle portion stain bright red, the head blue, and not infrequently with a pale-blue cap (Posner).

Florence's semen-reaction may also be mentioned here:—

A drop of potassium tri-iodide solution (iodin, 1.65; potassium iodid, 2.54; water, 30) is placed beneath a cover-glass with a drop of semen. At the periphery of the fluid there develop elongated *rhombic brownish crystals*, the formation of which is due to a certain stage of disintegration of lecithin. In freshly ejaculated semen this decomposition stage is physiologically present.

In **azoospermatorrhea** no spermatozoa are found in the thin, mucilaginous drops.

Prostatorrhea.—After a gonorrhea there not infrequently remains a chronic prostatorrhea which, from time to time, especially after frequent coitus, may lead to a thin or thick fluid, purulent discharge. Under such circumstances a recurrence of the gonorrhea might readily be assumed. The practitioner may more readily be led to such a con-

Fig. 134.—Spermatorrhea and Prostatorrhea. × 350.

s, Spermatozoa. *k*, Böttcher's crystals. *p*, Prostatic corpuscles. (Latter after *Bizzozero*.)

clusion when a drop of creamy secretion can be made to exude [at the meatus] by strong pressure [with the fingers] from the perineum forward. At other times the patient observes that such a drop appears on severe straining at stool or urination. This condition not infrequently coexists with spermatorrhea.

Microscopic examination is decisive in prostatorrhea. In such instances unquestionable cylindric epithelia are seen, and also colorless blood-cells, fat-droplets, frequent lamellated "amyloid elements," and very numerous Böttcher crystal octahedra, *which, according to Fürbringer's researches, are found exclusively in the prostatic juice and give to the semen its characteristic odor.*

Here, also, a most careful search for gonococci should be made. If they are absent in spite of the large numbers of pus-corpuscles present in the secretion, then, according to the author's firm conviction, supported by practice, the virulence of such a secretion can be excluded.

Azoöspermia.—In order to determine the procreative power of a male it is necessary in doubtful cases to examine for the presence of spermatozoa in the semen ejaculated during coitus (received in a condom). If permanent azoöspermia exists as a result of bilateral epididymitis, then the secretion, *which is*

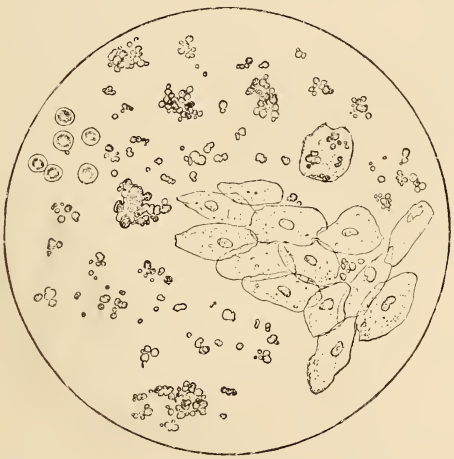


Fig. 135.—Hemoglobinuria.

exactly like the normal semen in quantity and odor, but often somewhat thinner and clearer, contains no sign of spermatozoa, but only a few round cells, epithelia, and octahedral crystals.

In **oligozoöspermia** the product contains a few spermatozoa, which show sluggish movements. In a case of this nature, although the patient has urinated freely before coitus, the author found in the condom contents, in addition to these feebly motile bodies and Böttcher's crystals, *a large number of pus-cells containing gonococci!*

In every *severe* hemoglobinemia produced by the different causes already referred to (page 184) there is an excretion of

blood-corpuscle residue in the urine, because the spleen and liver are not capable of consuming this (see page 185).

The *urine* is pale red to brownish black, and always decidedly diminished: sometimes to a few cubic centimeters in twenty-four hours. It is often voided only a drop at a time, and usually deposits a dense, brownish-red or blackish sediment composed of fine and coarse granules.

The specific gravity varies. It is usually low or normal, less often raised to 1.030. The albumin content is now and then considerable; usually, however, only a brownish-red, flocculent coagulum of albumin floats upon the surface. The exact nature of the albumin, which, according to Harley, is not serum-albumin, has not been determined. At all events, albumin may occur in the urine in hemoglobinemia *without* hemoglobin (Kast). The spectroscope shows the bands characteristic of oxyhemoglobin or methemoglobin (page 130, Fig. 70).

Microscopically the complete (or almost total) *absence of red blood-corpuscles* is especially worthy of attention. In addition to fine granular, brownish *detritus* the field of the microscope shows numerous small and large, pale or yellowish "droplets"; also coarse clumps of yellowish-brown color, and *especially in severe cases,* more or less long *cylindric* formations of a similar nature. Hematoïdin crystals or pigmented epithelia are rarely observed.

THE URINE IN OTHER CONDITIONS.

Scarcely half of the cases of **neoplasms** of the **kidney** and nearly all of those of the **bladder** are accompanied by hemorrhages. Discharge of characteristic carcinoma or sarcoma elements seldom occurs. In *tumors of the kidney cylindric, earth-wormlike blood-coagula are occasionally discharged.* These do not represent casts of the ureters, but are thus formed on their passage through this narrow duct. Their dark color speaks in favor of their not coming from a vesical hemorrhage. In a very characteristic case of malignant tumor of the kidney, confirmed by necropsy, the author saw numerous such wormlike formations removed from the patient by vesical irrigation. As regards the significance of "cancer-cells" and the diagnostic value of **fat-granule cells,** the reader is referred to remarks previously made (pages 243, 345, and 386).

The character of the urine voided by subjects affected with echinococcus, distomum, and filaria, as well as with urogenital tuberculosis, has already been discussed at length.

In **calculus formations** in the **renal pelvis** hemorrhages are more frequent than in neoplasms. The bleedings generally recur with colic. Catarrh of the renal pelvis usually coexists. The stones discharged from that location attain the size of a pea or bean and are often nodular.

Vesical calculi composed of urates, phosphates, and very rarely of cystin, frequently give rise to hemorrhages and mild muco-purulent catarrh, especially after bodily exertion. Ammoniacal urine is observed only after catheterization or other [mechanical] procedures.

Uric acid calculi are yellowish brown, smooth or slightly nodulated, and hard; oxalate calculi are much harder, mulberry-like, roughened, and usually dark in color. Phosphatic concretions are soft, finely roughened, and clayey. The stones are often composed of several bodies, and accurate chemic examination alone reveals the character and proportion of the individual earthy elements and organic matrix, which is always present, composing them (Ebstein).

APPENDIX: EXAMINATION OF THE SECRETIONS OF THE BREASTS AND VAGINA.

COLOSTRUM.

1. As is well known, a few drops of a whitish or whitish-yellow fluid can often by mild pressure be expressed from the mammæ of pregnant and puerperal women and those who have borne children. In addition to minute fat-droplets this fluid is microscopically characterized by the presence of fat-granule cells (colostrum-corpuscles). These resemble in every way the cells formed in "white kidney" (Fig. 115), and sometimes contain small, sometimes large, fat-droplets. They sometimes appear with and sometimes without a nucleus.

2. The fully formed **milk** presents a very uniform, fine emulsion without cellular elements.

3. In neoplasms of the mammæ a bloody discharge (from the healthy nipple) has been observed in rare instances.

EXAMINATION OF COWS' MILK.

[The following directions are given by Holt for the use of the apparatus shown in Fig. 136:—

"*The sample taken for examination* should be from the middle of the nursing or when the breast has been about one-half emptied, as the first milk is always poorer and the last richer than the average. About half an ounce is required.

"*The specific gravity* should be taken at a temperature of from 65° to 72° F. By giving the stem of the lactometer a twirl as it is introduced, it readily settles to the proper level, which may otherwise be prevented by the adhesion of the milk to the glass, especially in a rich specimen.

"The cylinder for *cream test* should be filled exactly to the top of the scale. After twenty-four hours the percentage of cream is read off, each degree in the scale being 1 per cent. A temperature ranging between 60° and 75° F. should be maintained.

"*Note* that the quantity of milk must be determined by other means; milk may be average in quality and very scanty.

"HUMAN MILK.

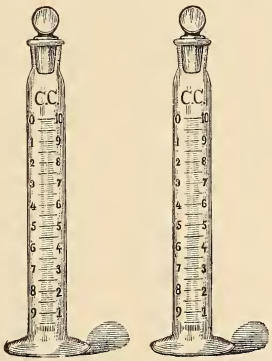
	SPECIFIC GRAVITY, 70° F.	CREAM, 24 HOURS	PROTEIDS
Normal average.	1.031	7%	1.5%
Healthy variations .	1.028 to 1.029	9 to 12%	Normal (rich milk)
Healthy variations .	1.032 to 1.033	5 to 6%	Normal (fair milk)
Unhealthy variations	Below 1.028	High (ab. 10%)	Normal or slightly below
Variations . .	Below 1.028	Nor. (5 to 10%)	Low
Variations	Below 1.028	Low (bel. 5%)	Very low (very poor milk)
Variations	Above 1.033	High	Very high (very rich milk)
Variations	Above 1.033	Normal	High
Variations	Above 1.033	Low	Normal (or nearly so)

"Milk presenting only moderate variations from the average—*e.g.*, specific gravity, 1.028; cream, 4 per cent.; or specific gravity, 1.033; cream, 10 per cent.—can usually be modified by appropriate treatment. If, however, the specific gravity is from 1.018 to 1.024 and the cream only from 2 to 3 per cent., it is hopeless."—BROOKS.]

For practical reasons the **examination of cows' milk** with the lactodensimeter and lactoscope is referred to in this place. The density is determined with the former, and with the latter the percentage of fat is estimated. Both methods of determination should always be made in conjunction.

1. The **density** of good (nonskimmed) milk should be, at 15° C., between 1.029 and 1.033.

The specific gravity is determined with the lactodensimeter (of Quevenne) by placing the spindle carefully into the cylinder containing a well-shaken sample of milk to be examined. The indicated density and the temperature, as shown by the accompanying thermometer, are carefully recorded. With the aid of a "correction table" the actual



[Fig. 136.—Holt's Milk Set.]

specific gravity can at once be obtained. If such a table is not at hand, the calculation must be made by adding to the specific gravity 1° for each 5° C. which the milk shows above the normal (15° C.) temperature, and *vice versa.*

2. The **amount of fat** in good market milk should not be less than 3 per cent.

The estimation with Feser's **lactoscope** is based upon the measurement of the degree of opacity of the milk, which is caused by the fat it contains (Fig. 137).

Procedure.—With the pipette *(P)* suck up the thoroughly mixed milk to the mark *M* and empty the contents of the tube into the cylinder *(C)*, at once washing the milk remaining in the pipette into the cylinder with some water. Then add water, while constantly shaking, until the

black marks upon the milk-glass *(A)* inside the cylinder are just made sufficiently distinct to be counted. The scale at the right at once gives the percentage of fat in the milk. The numbers on the scale at the left give the amount of water added in cubic centimeters.

[It is occasionally necessary to examine cows' milk for the presence of formalin, which has been added as a preservative. To this end use may be made of *Jorissen's reagent.* This consists of an absolutely colorless solution of 1 gram of phloroglucin in

Fig. 137.—Feser's Lactoscope.

1000 cubic centimeters of water and ordinary potash lye reduced to one-third strength. Place 25 cubic centimeters of the milk in a test-tube and add 10 cubic centimeters of the phloroglucin solution. After shaking and adding from 5 to 10 cubic centimeters of the potash lye, the presence of formaldehyd is shown by a salmon-red color, which disappears in a few minutes.

The Volumetric Determination of True Casein and Other Proteids in Milk by Dengiës's Method.—Twenty-five cubic centimeters of the milk are shaken with 1 cubic centimeter of a

30-per-cent. solution of potassium oxalate, and then treated with 20 cubic centimeters of a solution of 13.55 grams of mercuric chlorid and 36 grams of potassium iodid in a liter, followed by 2 cubic centimeters of glacial acetic acid. After dilution to 200 cubic centimeters the liquid is filtered, and 100 cubic centimeters treated with 10 cubic centimeters of potassium cyanid solution, equivalent to $\frac{N}{10}$ silver nitrate solution and 15 cubic centimeters of ammonium hydroxid, and titrated with $\frac{N}{10}$ silver nitrate solution until a permanent turbidity appears. The difference between the number of cubic centimeters

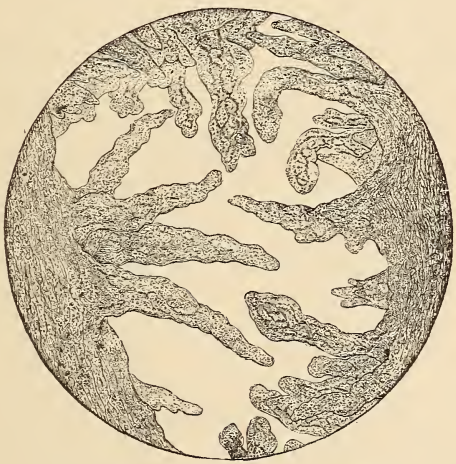


Fig. 138.—Chorionic Villi, from a Fresh Abortion (Low Magnification).

used and 48 gives a value corresponding with the total amount of proteids in grams per liter of milk.

To obtain the proteids not precipitated by acetic acid, 50 cubic centimeters of the milk are diluted with about 180 cubic centimeters of water, and after the addition of 0.2 cubic centimeters of glacial acetic acid, made up to 250 cubic centimeters and filtered, and the proteids in 125 cubic centimeters (= 25 cubic centimeters of milk) determined as above. The difference between the two results gives the amount of true casein in a liter of the milk.

Arnold and Mentzel's Method for the Detection of Hydrogen Peroxid in Milk.—As hydrogen peroxid is sometimes used as a preservative of milk, it may be necessary to test for its presence. The following method, which is suitable for the detection of this substance in both raw and heated milk, may be employed: Add to 10 cubic centimeters of the milk sample about 10 drops of a 1-per-cent. solution of vanadic acid in dilute sulphuric acid. A red coloration is produced by the presence of 0.01 gram of hydrogen peroxid in 100 cubic centimeters of milk. Titanic acid dissolved in dilute sulphuric acid gives a yellow coloration with 0.015 gram of hydrogen peroxid. The milk should be tested as soon as possible after it is received.

Detection of Tubercle Bacilli in Milk.—To 50 cubic centimeters of milk add 10 cubic centimeters of carbolic acid and shake vigorously for from two to five minutes. Then pour in a conic glass and let stand for twenty-four hours, or centrifuge to obtain sediment. Make preparations from the sediment as directed in sputum examinations, dry in the flame, wash for a few minutes in equal parts of alcohol and ether to remove fat, again dry in the flame, and stain by one of the methods given under "Sputum."—Brooks.]

EXAMINATION OF VAGINAL SECRETIONS.

Vaginal Secretions.—In the physiologic vaginal secretion there are found microscopically squamous epithelia and a variable number of leucocytes. As the result of numerous injuries the discharge may become more purulent, and, consequently, the microscopic picture be correspondingly altered.

In such cases the secretion is, under certain circumstances, to be examined for gonococci. According to prevailing views of gynecologists, however, a negative result in this respect is of no significance. Whether in the absence of gonococci it is justifiable almost invariably to refer the manifold disturbances (especially cases of pyosalpinx) to gonorrhea *is still a matter of doubt.*

The fact discovered by Döderlein—that the secretion of virgins is *always* and that of married women occasionally characterized by the presence of a special bacillus and an *acid* reaction, while cocci and an alkaline reaction are observed in the *majority* of such women in whom alterations have occurred in the vagina—is worthy of attention. Furthermore, the acid reaction is not caused by the bacilli alone, for even the *sterile* vagina of the healthy newborn always manifests an acid reaction.

The origin and nature of the acid have not been decided. At all events, the acid content appears to be of the greatest significance in the "self-purification" of the vagina, since, according to the investigations of Menge, the entrance and growth of bacteria in the vagina is always dependent upon the degree of acidity. "Large numbers of streptococci and staphylococci introduced into the vagina of newborn girls and adult women were more or less rapidly killed."

The wholly insignificant infusoria (cercomonas and trichomonas, page 105) are occasionally observed in the vaginal secretion.

Lochia.—The almost pure bloody *lochia rubra*, present during the first days after labor usually assume a flesh-water color *(lochia serosa)* on the third or fourth day; from the ninth day onward they are more gray or yellow-white *(lochia alba)*.

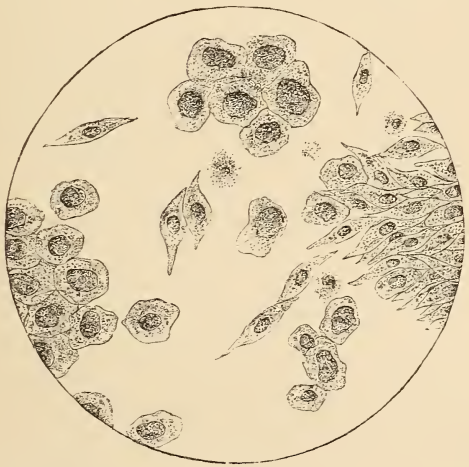


Fig. 139.—Decidual Cells, from a Fresh Abortion. × (about) 250.

In addition to numerous blood-corpuscles the microscope reveals in the first squamous epithelia and not infrequently remnants of decidua; in the later varieties numerous pus-corpuscles are observed which are in great part fatty; also fat-spherules and occasionally cholesterin crystals.

Abortion Hemorrhage.—A decision as to whether a blood-coagulum discharged from the vagina spontaneously or by artificial means contains remains of the ovum or not—a question of practical as well as forensic importance—can be greatly facilitated by the microscope.

If in such a blood-coagulum the chorionic villi illustrated in Fig. 138 are found, in which, in addition to a capillary reticulum,

more or less advanced fatty degeneration can, not infrequently, be made out, this alone suffices to assure the diagnosis of hemorrhage of pregnancy.

Furthermore, the detection of *decidual cells*—which are characterized by their size; round, polygonal, or spindle-shaped contour; and usually very distinct nucleus and nucleolus (Fig. 139)—is of importance. The preparations can be easily made by teasing minute fragments.

VI. EXAMINATION OF ASPIRATED FLUIDS.

The microscopic examination of aspirated fluids supplements to a marked degree the macroscopic findings. It often reveals certain elements which not infrequently furnish the first evidence of the character of the essential lesion. Both of these methods of examination should, therefore, be conducted in conjunction.

The author very frequently practices exploratory puncture, and in innumerable instances he has been greatly aided in diagnosis thereby. If the skin is always carefully cleansed with ether and a sterilized needle is employed, infection, which has brought this method of examination into discredit with many physicians, need not be feared. It is true that, even at the present day, many unusual things may be experienced; asepsis is still defective in many respects! Especial precaution is necessary in punctures in the region of the kidney and in enlargements of the liver. In spite of numerous exploratory punctures in these regions, the author has never experienced bad results. Nevertheless, three cases of fatal intraperitoneal hemorrhage are known to him which were caused by puncture of enlarged livers. In all three instances, however, severe infectious suppuration of the gall-bladder and bile-ducts, with intense icterus, were present. Apparently the coexistent blood-decomposition favored the unfortunate result.

1. TRANSUDATES.

Transudates originate without inflammatory processes. They are usually translucent, light yellow in color, with a slight-greenish tinge, alkaline in reaction, and on standing deposit a flocculent coagulum which is usually scanty. Their specific gravity at room temperature (not in fluids at the temperature of the body) varies according to their origin. According to the careful researches of Reuss (from the clinic at Tübingen) it is:—

In hydrothorax lower than	1.015
In ascites lower than	1.012
In anasarca lower than	1.010
In hydrocephalus lower than	1.0085

In pleuritis it varies between 1.017 and 1.027, and in peritonitis between 1.016 and 1.022.

As the specific gravity of these fluids chiefly depends upon their albumin content, the latter, according to Reuss, can be determined with tolerable certainty from the former. In the serofibrinous exudates of the pleura the albumin content is almost never below 4.5 per cent.; those of the peritoneum, 2 to 2.5 per cent. In transudates of the pleura it is always below 2.5 per cent., and of the peritoneum between 1.5 and 2 per cent.

Microscopically a few leucocytes, usually in a state of fatty metamorphosis, and occasional normal squamous epithelia are found.

2. EXUDATES.

Exudates originating as the result of **inflammatory processes** present many variations. According to their external appearance we distinguish *serous* (serofibrinous), *hemorrhagic, purulent,* and *ichorous* exudates, and the mixed forms resulting from commingling of these chief constituents.

The specific gravity of all of these is above 1.018, and the reaction is always alkaline. On standing they deposit more or less fibrin, and above this a bloody, purulent, or ichorous sediment.

In *rare instances* a progressive, *dark-blue discoloration* of a previously transparent yellow exudate is observed. In such cases the coloring matter is at first contained in the exudate as a so-called *leuco-product* and develops into the *blue* body only on oxidation (exposure to air). By reduction (addition of strong alkaline solution of grape-sugar) it can be rendered invisible and be made to reappear in the yellowish solution by addition of a few drops of pure hydrochloric acid and dilute solution of iron chlorid. Addition of fuming sulphuric acid causes separation of deep-blue indigo-sulphate.

In melanotic carcinosis a cloudy, *dark-brown fluid* may be discharged in which a *blackish sediment* is deposited.

Sometimes a peculiar glistening, iridescent pellicle is observed upon the surface of aspirated fluids obtained from old pleuritic exudates. This shimmer is due to the presence of cholesterin (see page 210).

Serous Exudates.—The yellow, translucent fluid, which is slightly cloudy immediately after evacuation, sooner or later throws down slightly flocculent or dense coagula, which not infrequently show a faint-reddish streak.

Microscopically a dense fibrin reticulum is found in the flocculent coagula; a few red blood-cells, which are chiefly derived from the puncture itself, are always observed, and likewise numerous multinucleated leucocytes with a greater or less amount of usually finely or coarsely granular protoplasm. Not infrequently these cells are considerably enlarged, and are then scarcely distinguishable from *pleura endothelia.*

Hemorrhagic Exudates.—Sero-fibrinous exudate is colored dark or light red by the rich admixture of blood. Microscopically, the same elements observed in the former variety are found in it, but, of course, the red blood-cells are present in much larger number. The latter are, for the most part, well preserved, though in chronic exudates they are in part "leached," or decolored.

As bloody exudates, aside from existing hemorrhagic diathesis and traumatism, most frequently occur in tuberculosis and neoplasms, their occurrence is of important diagnostic and prognostic value. Thorough microscopic examination of the sediment must, therefore, never be neglected, because it often furnishes valuable information for a positive diagnosis.

Demonstration of *tubercle bacilli* is rare, even *after* centrifugation of the aspirated fluid.

In cases of *carcinoma* it is sometimes possible to find peculiar cell-formations or even villi. In a case under the author's observation, which has been published by Dr. Harries, the fluid was mixed with *countless colloid masses.*

In previous chapters of this work the author has repeatedly warned against the diagnosis of "cancer-cells." However, as we consider the frequent occurrence of massed groups of epithelial elements in vesical cancer as of diagnostic value, *we must also, in the present instance, emphasize the importance of the abundant appearance of large cells manifesting marked morphologic variations.*

In cases of *neoplasms the cells may often attain extraordinary size, even as large as 120 μ,* and they are usually character-

ized by one or more vacuoles and generally lie in groups. They contain a single large nucleus or, less often, several nuclei, and almost always small and large fat-droplets, the close accumulation of which may give rise to enormous "fat-granule corpuscles" [Gluge's corpuscles]. The diagnostic value of these cells has already been fully referred to on pages 243 and 345 and illustrated in Fig. 97.

In addition to such cell aggregations the occurrence of numerous *large free fat-droplets* should arouse suspicion of neoplasm. Sometimes the fat-droplets are so minute and suspended in the fluid in such large numbers as to give to the latter a *chylous* appearance. If this is the case, the milky character disappears on addition of sodium hydrate and shaking with ether. In other cases, however, the chylous fluid is not rendered clear by this procedure, which is proof that the opalescence is not due to emulsified fat, but to fine albuminous granula (Quincke).

In a case of carcinoma of the serous membranes observed by the author recently in Hamburg, he found on repeated aspiration of the left pleura and peritoneal cavities a pure chylous, and in the right pleural cavity a sero-hemorrhagic, exudate. The necropsy gave no explanation of this difference. The escape of chyle was caused by carcinosis of the thoracic duct.

Sometimes the rich occurrence of *rosette-formed masses of fine fat-needles,* 20 to 30 μ in size, is worthy of attention (see Fig. 41). The author found such in large numbers in an exploratory puncture, which he made in a case of secondary pleuritis consecutive to carcinoma of the bronchi which was confirmed at necropsy.

In *primary endothelial carcinoma of the pleura* (E. Wagner) the abundant occurrence of polymorphous cells and *fat-granule spherules* has also repeatedly been observed.

In a case reported by A. Fränkel, in 1892, aspiration showed a dark-red, cloudy fluid resembling *venous* blood, which contained numerous, large round or exquisite polymorphous, polyhedral, squamous, epithelioid cells and club-shaped and caudate varieties. In addition to a large nucleus and vacuoles many of them showed—by virtue of their rich content of fat-droplets—a decided mulberry shape (apparently fat-granule spherules; see Fig. 97). At necropsy (six weeks after beginning of the affection) Fränkel found, not the expected pleural carcinoma, but the above-mentioned affection, which, from the very first, is especially

characterized by a pronounced tendency to diffuse extension by way of the lymph-channels. According to Neelsen, this peculiarity makes the assumption of an infectious inflammation more probable than of a tumor-formation. The author himself, in a similar, but more chronic, case, based a diagnosis especially upon the findings in the aspirated fluid. The necropsy showed that the whole left pleural cavity was occupied by an endothelial carcinoma.

Villous fragments or **colloid masses** and other constituents of the neoplasm, however, render a diagnosis absolutely certain. In a case of peritoneal carcinoma observed by the author, *innumerable, soft, elastic, translucent, colloid masses, about the diameter of a lentil or a pea,* were found in the hemorrhagic exudate removed with an ordinary Billroth trocar. At first these were thought to be small echinococcic vesicles, but microscopic examination of fresh "crush" preparations at once corrected the error. They presented an exquisite alveolar structure [of colloid carcinoma].

[A similar case was recently admitted to the New York Post-graduate Hospital, in the service of Prof. S. S. Burt, in which the pinkish fluid withdrawn by cannula from the peritoneal cavity showed numerous colloid masses. Microscopic examination of these masses showed the characteristic alveolar structure of colloid carcinoma and at once established the diagnosis. The case subsequently came to necropsy, which revealed colloid cancer of the stomach and omentum, and confirmed the microscopic findings. A history of the case was published in the *Zeitschrift für Praktische Aerzte,* 1 and 2, 1902.—Brooks.]

On staining with hematoxylin and eosin a reticulum, composed of connective-tissue bands, appeared surrounding irregular alveoli. Some of the alveoli were filled at the periphery with cylindric epithelia, but the majority of them contained irregularly distributed, round, elongated, or branching cells. In the areas of advanced degeneration, which occurred from the center toward the periphery [of the alveolus], the contents consisted of a granular, red, mucoid mass, which presented distinct striations running parallel to the wall of the alveoli, with here and there nuclei or isolated round or cylindric cells filled with finely granular protoplasm. The necropsy showed an unusually extensive colloid carcinosis of the omentum.

According to the author's experience, exploratory puncture may be of decided value in firm tumors involving, for example,

the upper lobes of a lung, and reveal the above-mentioned elements.

Cholesterin crystals are occasionally met with in sero-hemorrhagic exudates present in *chronic pleuritis.* Attention is directed to them by a peculiar *shimmer* upon the surface of the fluid. This appearance, however, is quite infrequent, for, among several hundred exploratory punctures of the pleural cavity, the author met with it in only a few cases. The crystals are readily identified by their characteristic form of crystallization and their chemic peculiarities.

Hemosiderin granules and clumps are quite frequent in chronic hemorrhagic exudates.

Purulent exudates are more or less deep yellow in color and deposit a corresponding sediment of pus. They usually present no especial microscopic peculiarities. Examination for **bacteria** is particularly important. For this reason, in addition to inspection of the fresh pus, which usually shows fatty-degenerated pus-corpuscles, *it is always advisable to stain dry preparations.* In tuberculous exudates (pneumopyothorax) *tubercle bacilli* are very rarely found. [One case has recently come under our observation in which considerable numbers of these bacilli were readily found in a pleural exudate.—Brooks.] In other exudates, however, staphylococci, streptococci, and Fränkel's pneumococci are found, the latter almost invariably in metapneumonic empyema. In a nonputrid pneumothoracic exudate Litten repeatedly found numerous *cercomonas* forms. *Empyemas which are found free of micro-organisms are almost always tuberculous in character.*

[The effusion of primary tubercular pleurisy contains almost exclusively small lymphatics. In hydropneumothorax, cancerous or ulcerating lesions of the lungs, numerous polynuclears are found, while in mechanical pleurisy the endothelial cells of the pleura predominate (Roger). In streptococcic pleuritis neutrophilic polynuclears are almost exclusively present. In pneumococcic pleuritis the majority of the cells are polynuclears and large mononuclears mixed with erythrocytes, and a few lymphocytes.—Brooks.]

In every *obscure case actinomyces* should also be looked for and the purulent deposit very carefully scrutinized (upon a porcelain dish or glass plate) for the *fungous granules.* These

appear as small, gritty particles of chalklike consistence, which can usually readily be crushed beneath a cover-glass (Fig. 39). In addition to these bodies, distinct fat-granule corpuscles are often observed.

Putrid exudates occur in the pleural as well as in the peritoneal cavity as the result of perforation of gangrenous foci, or from gastric or intestinal ulcers and neoplasms, and sometimes without distinct cause. The aspirated fluid often emits a stinking odor; the presence of sulphureted hydrogen is recognized by the dark discoloration of the cannula. If a serous exudate is

Fig. 140.—Sodium Chlorid Crystals Produced by Careful Evaporation of Echinococcic Fluid. × 350.

met with on aspiration of an upper intercostal space and a putrid exudate in a lower, subphrenic abscess should be suspected.

In such instances, in the author's opinion, the presence of the *bacillus coli communis* should be looked for more often than has heretofore been the custom. The author met a case of large exudate (located in the left [!] pleural cavity) which, in addition to air, contained chiefly abundant bile-tinged fluid with moderately feculent odor. Bacteriologic examination showed pure culture of *bacillus coli communis.*

In perforation of a *gastric ulcer* the aspirated fluid may contain *yeast* and *sarcina fungi,* and the *reaction* of the *exudate* may be *acid.*

3. ECHINOCOCCIC CYST CONTENTS.

Echinococcic cyst contents is perfectly clear and nonalbuminous. In addition to succinic acid it contains chiefly sodium chlorid, which, on slow evaporation of a drop of the fluid upon a glass slide, crystallizes in the forms shown in Fig. 134. The specific gravity varies between 1.008 and 1.013.

Frequently no trace of morphologic elements is found on **microscopic examination.** Sometimes only a few hemosiderin granules or cholesterin crystals and isolated fatty-degenerated cells are observed. Not infrequently, however, the scolices, hook-

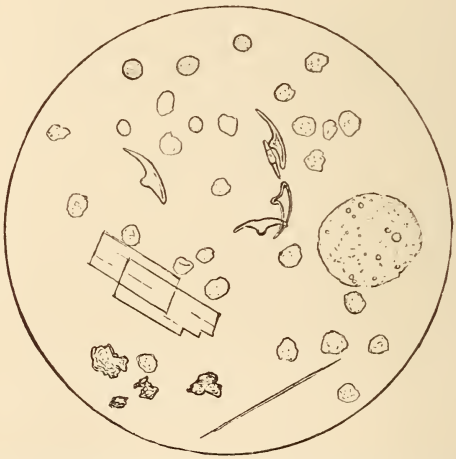


Fig. 141.—Echinococcic Hooklets, Obtained by Aspiration of a Cyst. × 350.

lets, or membrane fragments offer absolute evidence (Fig. 141). The crystals shown in Fig. 140 can almost always be demonstrated. On a number of occasions, however, the author has obtained an opalescent and very cloudy fluid by aspiration. In these instances the cysts had remained unrecognized up to that time, and they certainly had not been punctured before. Microscopically there were found various bacteria, among them pyogenic cocci; also invariably cholesterin crystals, hooklets, and shreds of membrane. There is no doubt that such formations as are shown in Fig. 142 can also occasionally be seen in the

aspirated fluid. The preparation from which the illustration was taken was obtained from a superficial hepatic cyst, about the size of the fist, which was accidentally discovered in a cadaver. Ten cubic centimeters of fluid with the interesting contents was obtained by aspiration. [Several years ago, in a case of echinococcic cyst of the liver involving the spinal canal and presenting symptoms of paralysis, I observed in the perfectly clear fluid aspirated from the side of the spine a number of motile scolices like those shown in Fig. 142. The case occurred in the practice of Prof. Samuel Lloyd during his service in the surgical wards of the New York Post-graduate Hospital.

Fig. 142.—From an Echinococcic Cyst. × 350.

The case was subsequently reported by him in the *American Medico-Surgical Bulletin*, December 5, 1896. By an oversight, credit for the discovery of these bodies was given to Dr. W. D. Bullard, then house surgeon, to whom they were demonstrated by me.—Brooks.]

If the fluid is characterized by a large amount of *cholesterin*, a decided *shimmer*, due to the agglomerated crystals, can be noted even with the naked eye.

Chemic Test.—In addition to tests for albumin, the demonstration of *succinic acid* is in some instances desirable. Some of the fluid is acidulated with HCl, evaporated, and then shaken with ether. If succinic acid is present, the semifluid crystalline mass left after evaporation of the

ether gives in watery solution a rusty, gelatinous precipitate (succinate of iron) with iron chlorid.

4. OVARIAN CYSTS.

The usually tenacious, slimy contents has a very variable specific gravity. It may range from 1.005 to 1.050, but generally lies between 1.020 and 1.024. As a rule, it is strongly albuminous and rich in *metalbumin,* which is not precipitated by either acetic acid, nitric acid, or boiling. On addition of alcohol, however, the metalbumin is precipitated in a flocculent state, in which respect it differs decidedly from mucin. In performing the test *the albumin must first be removed* (see "Urine").

The cyst contents, which is usually yellow in color, may occasionally present a dark-red or chocolate hue.

Red and colorless blood-cells, and not infrequently blood-pigment and cholesterin, quite often fat-granule cells, compound granular cells [Gluge's corpuscles], and large, vacuolated cells are found on microscopic examination.

The presence of cylindric epithelial, ciliated, and goblet-cells, as well as *colloid concretions,* is emphasized by Bizzozero as of considerable importance. "The latter are from a few μ to one-tenth millimeter in size, irregularly formed, homogeneous, and pale yellow in color, and are distinguished from fat and calcareous substances by their paleness."

5. HYDRONEPHROSIS.

The author has obtained the contents of acute and subacute hydronephrotic cysts at least a dozen times by aspiration and thus secured important information for diagnostic and therapeutic purposes. Calculi, stenoses, unknown causes, and especially trauma had produced the affections. The usually water-clear, less often cloudy, reddish, or dirty-yellow contents are also distinguished from ovarian cystic fluid by a low specific gravity, which is constantly below 1.020 (usually between 1.010 and 1.015). Furthermore, urea and uric acid (see page 283) and only slight amounts of albumin are generally found. It should be remembered that the urinary constituents may be absent in old cysts, and that slight amounts of uric acid may be present in ovarian cysts.

The microscopic findings are, as a rule, extremely meager. Organized elements, such as epithelia from the kidneys and urinary passages, are but seldom observed; these have already been fully described. Usually only red and colorless blood-cells are seen.

In *renal tumors*, also, aspiration may occasionally aid in diagnosis. In a case of an enormous tumor of the left kidney, almost as large as a man's head, upon the nature of which many dissenting professional opinions had been expressed, the author obtained by aspiration, in addition to peculiar tumor-cells, characteristic *renal tube-casts*, whose occurrence left absolutely no doubt as to the origin of the tumor. Extirpation revealed an enormous adenoma of malignant type.

6. HYDROPS OF THE GALL-BLADDER.

Exploratory aspiration, which is usually *not* to be commended, sometimes shows only a clear mucoid or serous fluid. In inflammatory processes a more or less large number of colon bacteria are usually present. In chronic biliary congestion the bacilli cause an infectious angiocholitis, and may very probably give rise to stone-formation by decomposition of the bile and deposition of precipitates of bilirubin-calcium around the luxuriantly developing masses of bacilli. In empyema the pus often has an offensive odor.

7. ASPIRATION OF SPINAL CANAL.

Owing to its diagnostic value, this method, first recommended by Quincke, is deserving of consideration in this place.

The patient is placed upon the side with the spine arched well outward. A fine, hollow needle is introduced into the spinal canal at a point below the spinous process of the second and third lumbar vertebræ, exactly in the middle line, and the fluid allowed to escape by internal pressure. If the internal pressure is increased (500 to 700 millimeters of water), the fluid will at first flow in an arched stream. At other times it will escape only in drops even at the beginning. At one sitting from 20 to 100 cubic centimeters may be obtained.

According to the author's experience, based upon a hundred spinal punctures, the following features are of importance:—

1. **In Tuberculous Cerebrospinal Meningitis.**—The fluid, which, with variable exceptions, is always abundantly obtained

under high pressure, is usually water-clear, much less frequently somewhat opalescent. In it there often separates a delicate, spiderweblike membrane in which the tubercle bacilli are most likely to be found. The finding of tubercle bacilli is possible, however, in only half of the cases. The fluid almost always contains a large number of *leucocytes*. The *specific gravity varies between 1.005 and 1.011; the proteid content* is seldom found to be less than 0.5 per mille with Esbach's, usually 2 to 3, but it may be as high as 12 per mille.

2. **In Acute Nontuberculous Forms of Meningitis.**—Here, also, especially in that form caused by *Weichselbaum's coccus,* the fluid may be clear. More frequently, however, it is somewhat opalescent. Sometimes it is slightly or even densely purulent. The latter is much more common in streptococcic meningitis.

The specific gravity and albumin content vary about as in the preceding section, No. 1.

In **acute primary** cerebrospinal meningitis there is found in the exudate, in addition to the small diplococcus *intra*cellularis, which also occasionally occurs outside the cells (see page 43), most frequently Fränkel's diplococcus. The question which coccus is to be considered as the true cause of *epidemic* cerebrospinal meningitis is not yet settled. Many observers, among them the author, incline to the opinion that Weichselbaum's coccus is the specific etiologic factor. The author, however, does not think an absolute decision in this regard is possible, because in more than two dozen cases of primary meningitis he found Weichselbaum's coccus in only 60 per cent. and Fränkel's coccus (exclusively) in about 30 per cent.

In Weichselbaum's meningitis the characteristic bacteria (see page 43) are very rarely missed. In the streptococcic form a long search for the bacteria is often at first negative. This is apparently due to the fact that an intense inflammatory irritation, manifested by secretion of large numbers of leucocytes, starts from an (otitic or other) abscess-focus, the germs appearing only after true rupture of the abscess has occurred.

Only in the rarest instances are other bacteria—*e.g.,* typhoid bacilli (the first case of this kind was reported by the author in 1887, at the International Congress at Berlin)—to be found as exciters of the meningitis.

In **chronic** pachymeningitis the aspirated fluid is usually clouded by blood.

3. In severe **chlorosis** accompanied by violent headache, the latter can, not infrequently, be entirely dissipated by lumbar puncture. In such instances the pressure will be found to be greatly augmented (300 to 450 millimeters) and the fluid increased to such a degree that from 20 to 30 and even 50 cubic centimeters quickly escape.

4. In **apoplexy** a rupture into the lateral ventricles may be diagnosticated from the pure bloody character of the aspirated fluid; while in severe injuries to the skull the absence of admixture of blood may, under certain circumstances, justify the diagnosis of an extradural hemorrhage.

5. In **cerebral tumors** the water-clear fluid is *rarely* rich in leucocytes or albumin. It usually contains only traces of these. The author, however, has seen a *few* cases (confirmed at necropsy) with high albumin content. In addition, small amounts of sugar are occasionally found.

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PLATE VII

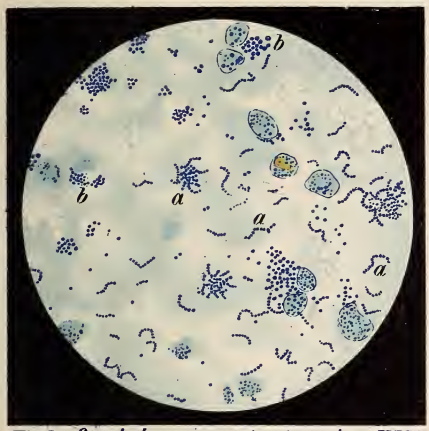

Fig.1. Staphylococci and streptococci. X750.

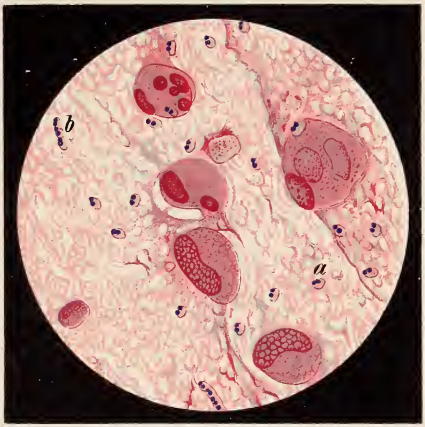

Fig.2. Diplococcus pneumoniae. X750.

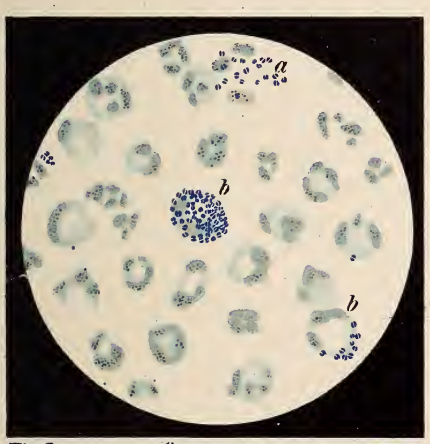

Fig.3. Gonococcus. X750.

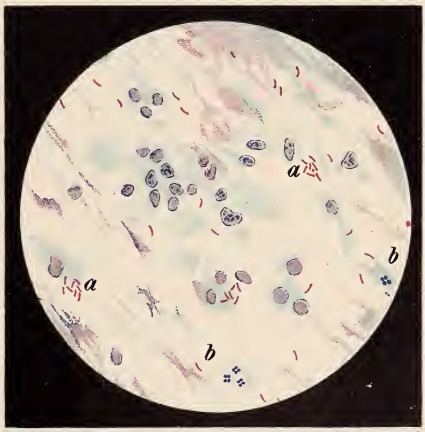

Fig.4. Tubercle bacilli (sputum) X750.

Fig.5. Anthrax bacilli. X500.

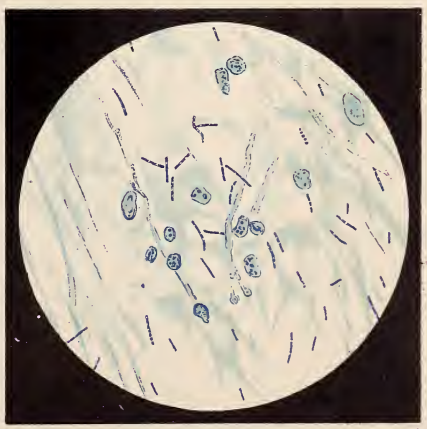

Fig.6. Glanders bacilli. X750.

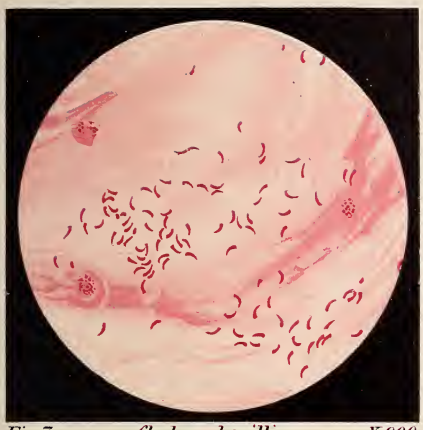

Fig. 7. *Cholera bacilli.* *X 800.*

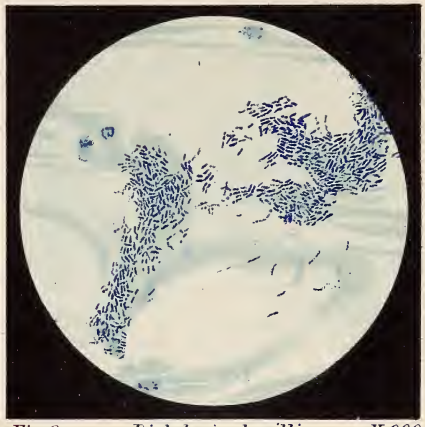

Fig. 8. *Diphtheria bacilli.* *X 800.*

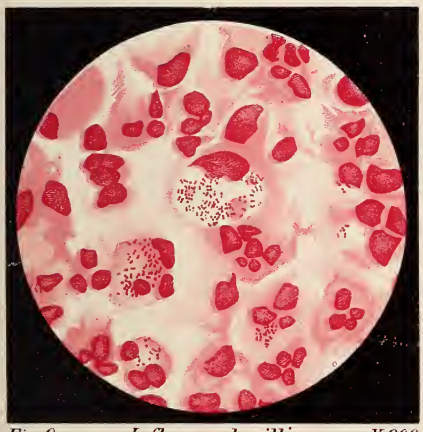

Fig. 9. *Influenza bacilli.* *X 800.*

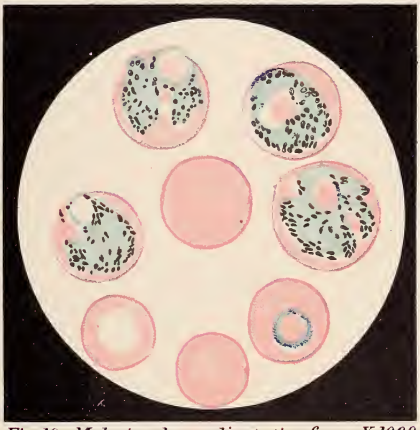

Fig. 10. Malaria plasmodia, tertian form. X 1000.

Fig. 11. Malaria plasmodia, tropical form. X 1000.

Fig. 12. Malaria plasmodia, tropical form. X 1000.

Fig.13. Progr. pernicious anemia. X750.

Fig.14. Splenic leukemia. X750.

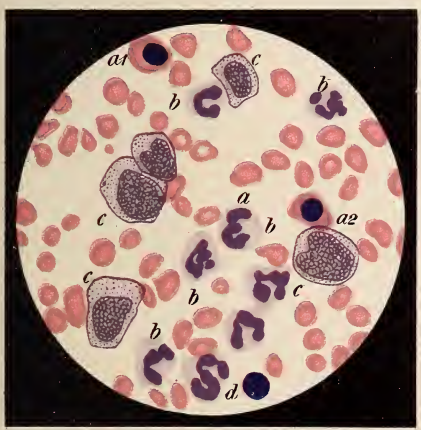

Fig.15. Splenic leukemia. X800.

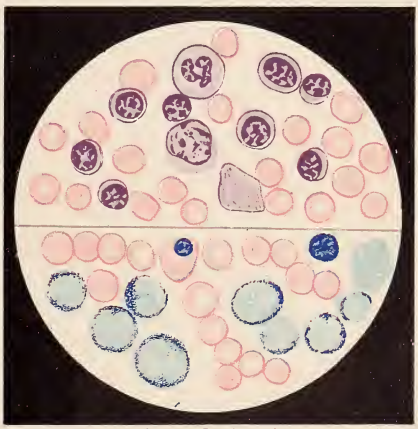

Fig.16. Acute leukemia. X800.

Fig.17. Hemin crystals. X600.

Fig.18. Heart lesion cells. X.600.

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